

T.R.

GEBZE TECHNICAL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

**QUATERNIZED AMMONIUM FUNCTIONALIZED POLY(LACTIC ACID)
FILMS:STRUCTURE-ANTIMICROBIAL ACTIVITY RELATIONSHIP AND
MECHANICAL/BARRIER PROPERTIES**

FİGEN AYNALI

A THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMICAL ENGINEERING

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GEBZE

2020

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GEBZE TEKNİK ÜNİVERSİTESİ
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**KUATERNER AMONYUM GRUBU İÇEREN POLİ(LAKTİK ASİT)
FİLMLERİ: YAPI-ANTİMİKROBİYAL ETKİ İLİŞKİSİ VE
MEKANİK/BARİYER ÖZELLİKLERİ**

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2020

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SUMMARY

In the present thesis, poly(lactic acid) based films having antimicrobial activity were prepared. For this purpose in the first step, biodegradable PLA copolymers bearing azido groups were synthesized via the ring opening copolymerization (ROC) of L-lactide and 2,2- bis(azidomethyl)trimethylene carbonate cyclic monomers. Then, alkyne functionalized quaternary ammonium salts were attached onto the azido groups of these copolymers using copper(I)-catalyzed azide alkyne cycloaddition (CuAAC) to impart antimicrobial activity to the copolymers. The main aim was to obtain antimicrobial polymers that antimicrobial agents were attached via covalent bonds. The antimicrobial activity tests of these synthesized polymers were carried out against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria by the drop plate method. It was observed the polymers exhibited highly effective antimicrobial activity. Also, the activity depended on the compositional ratio and the length of the alkyl group on the ammonium salts. In the second step, the synthesized copolymer containing the largest amount of antimicrobial agent was employed to coat one side of neat PLA film in three different proportions. After the coating process, the thermal, mechanical and barrier properties of the films were investigated and that was determined the coating process had no negative effect on these properties of the films.

As a result, it was aimed to synthesize PLA films having antimicrobial agents which are bound covalently and films with desired properties were obtained. These PLA based antimicrobial films are thought to have potential to be used in broader fields of applications such as food packaging, biomedical devices and implants.

Key Words: Click Chemistry, Ring-Opening Copolymerization, Poly(lactic acid), Antimicrobial Film, Spray-Coating.

ÖZET

Bu tez çalışmasında antimikrobiyal özelliğe sahip poli(laktik asit (PLA) filmleri hazırlanmıştır. Bu amaçla, öncelikle azid fonksiyonlu 2,2- bis(azidometil)trimetilen karbonat ve L-lactide monomerlerinden halka açılma polimerizasyonu yöntemi kullanılarak kopolimerler sentezlenmiştir. Daha sonra, antimikrobiyal etkiye sahip PLA elde edilmesi amacıyla alkin fonksiyonlu kuaterner amonyum tuzları CuAAC metodu kullanılarak kopolimerdeki azid gruplarına bağlandı. Bu polimerlerin Gram-negative (*Escherichia coli*) ve Gram-positive (*Staphylococcus aureus*) bakterilerine karşı antimikrobiyal aktiviteleri besiyeri yüzeyine damlatma yöntemi ile test edildi. Sentezlenen polimerlerin oldukça etkili antimikrobiyal etki gösterdiği görüldü. Ayrıca, aktivite orana ve amonyum tuzlarındaki alkil gruplarının uzunluğuna bağlıydı. İkinci aşamada ise çözücü-döküm yöntemi kullanılarak hazırlanan PLA filminin bir yüzeyi, yapısında en fazla oranda antimikrobiyal ajan içeren kopolimer ile farklı oranlarda kaplandı. Elde edilen filmlerin *S. Aureus* bakterisine karşı aktivitesi direkt-temas yöntemi ile test edildi. Ve, filmlerin en düşük kaplama oranında dahi oldukça etkili antimikrobiyal aktivite gösterdiği görüldü. Bu durum kullanılan kimyasal miktarını oldukça azalttığından hem sağlık hem de ekonomik açıdan önemli avantaj sağlamaktadır. Ayrıca, kaplama işlemi sonrası filmlerin termal, mekanik ve bariyer özellikleri araştırıldı ve yapılan işlemin filmlerin bu özellikleri üzerine olumsuz etkisi olmadığı saptandı.

Bu tez kapsamında elde edilen antimikrobiyal filmlerin gıda paketlenmesi, biyomedikal aletler ve implantlar gibi alanlarda kullanım potansiyeline sahip olabilecekleri düşünülmektedir.

Anahtar Kelimeler: Klık kimyası, Halka Açılma Kopolimerizasyonu, Poli(laktik asit), Antimikrobiyal Film, Sprey-Kaplama.

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LIST of ABBREVIATIONS and ACRONYMS

<u>Abbreviations and Acronyms</u>	<u>Explanations</u>
δ_p	: Solubility parameter
ΔH_f	: Enthalpy of Crystal Fusion
ADTC	: 2,2-bis(azidomethyl)trimethylene carbonate
AFM	: Atomic Force Microscopy
ASTM	: American Society for Testing and Materials
CaH_2	: Calcium Hydride
CAS No	: Chemical abstracts servis number
CO_2	: Carbone dioxide
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	: Copper sulfate pentahydrate
CuAAC	: The Copper(I)-catalyzed azide-alkyne cycloaddition
DCM	: Dichloromethane
DLS	: Dynamic Light Scattering
DMF	: <i>N,N</i> -Dimetilformamide
DMSO	: Dimethyl Sulfoxide
DSC	: Differential Scanning Calorimetry
E	: Young's (Elastic) modulus
E%	: Elongation at Break
EDTA	: Etilendiamin tetraasetik asit
EDX	: Energy dispersive X-ray
FDA	: American food and drug administration
FT-IR	: Fourier Transform Infrared
GPC	: Gel Permeation Chromatography
GRAS	: Generally recognized as safe
GTA	: Glycerol triacetate
LA	: Lactic acid
M_n	: The number-average molecular weights
$M_{w,th}$	: The theoretical molecular weight

PDI (M_w/M_n)	:	Polydispersity Index
MFR	:	Melt Flow Rate
MIC	:	minimum inhibitory concentration
MMT	:	Modified montmorillonite
MW	:	Molecular Weight
NaN ₃	:	Sodium azide
NMR	:	Nuclear Magnetic Resonance
OTR	:	Oxygen Transmission Rate
OP	:	Oxygen Permeability
PA	:	Polyamide
PBAT	:	Poly(butyleneadipate-co-terephthalate)
PC	:	Polycarbonate (PC)
PCL	:	Polycaprolactone
PDLA	:	Poly(DL-Lacide)
PE	:	Polyethylene
PEG	:	Poly(ethylene glycol)
PET	:	Poly(ethylene terephthalate)
PGA	:	Polyglycolide
PHAs	:	Polyhydroxylalkanoates
pKa	:	The acid dissociation constant at logarithmic scale
PLA	:	Poly(lactic acid), Polylactide
PLA (Ref)	:	Neat PLA obtained via solvent casting method
PLA+5	:	PLA coated with antimicrobial polymer 5 times
PLA+15	:	PLA coated with antimicrobial polymer 15 times
PLA+25	:	PLA coated with antimicrobial polymer 25 times
PLGA	:	L-Lacide-glycolide copolymer
PLLA	:	Poly(L-lactide)
PMDETA	:	<i>N,N,N',N'',N'''</i> - Pentamethyldiethylenetriamine
PMMA	:	Poly(methyl methacrylate)
PP	:	Polypropylene
PS	:	Polystyrene

PTT	:	Poly (trimethylene terephthalate)
PVC	:	Polyvinylchloride
PVOH	:	Polyvinyl alcohol
PQAs	:	Polyquaternary ammoniums
ROP	:	Ring Opening Polymerization
SEM	:	Scanning Electron Microscope
Sn(Oct) ₂	:	Tin(II) bis-(2-ethylhexanoate)
T _{d,0}	:	Initial Decomposition Temperature
T _{d,1/2}	:	Half Decomposition Temperature
T _{d,onset}	:	The Onset Temperature
T _{d,max}	:	The Thermal Decomposition Temperatures
TEA	:	Triethylamine
TEM	:	Transmission Electron Microscopy
T _g	:	The Glass Transition Temperature
TGA	:	Thermal Gravimetric Analysis
TMC	:	Trimethylene Carbonate
T _m	:	The Melting Point
THF	:	Tetrahydrofuran
TS	:	Tensile strength
V _c	:	Molar volume of semicrystalline polymer
V _g	:	Molar volume of glassy amorphous
V _w	:	Van der Waals volume
WVP	:	Water Vapor Permeability
WVTR	:	Water Vapor Transmission Rate
QAS	:	Quaternary Ammonium Salts
QAS-12C	:	<i>N,N</i> -dimethyl- <i>N</i> -prop-2-yn-1-yl dodecane-1-ammonium bromide
QAS-6C	:	<i>N,N</i> -dimethyl- <i>N</i> -prop-2-yn-1-yl hexane-1-ammonium bromide
QAS-3C	:	<i>N,N</i> -dimethyl- <i>N</i> -prop-2-yn-1-yl propane-1-ammonium bromide
X _c %	:	The Percent Crystallinity

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1. INTRODUCTION

Almost all polymeric materials currently used are produced from non-renewable fossil resources. They are resistant to degradation, and as a result their disposal has become a serious question when used specially in areas such as pharmacology, surgery, agriculture, the environment, etc. [1]. In response to these concerns, great attention is paid to biodegradable polymers derived from biomass (non-fossil origin) materials to be used as packaging materials and containers due to reduction of greenhouse gas and toxic emissions, and for medical applications because of their biodegradability, biocompatibility and non-toxicity. Poly(lactic acid) (PLA) is one of the most promising materials among the renewable source-based plastics because it is not only biocompatible and biodegradable, but it is a thermoplastic aliphatic polyester [2]. PLA can be synthesized by condensation polymerization of lactic acid monomers (LA), also named 2-hydroxy propionic acid, or by the ring opening polymerization of cyclic lactide monomer [3], [4]. Both types are commonly referred to as PLA. The most common way to obtain high-molecular-weight PLA is ring opening polymerization (ROP) of the lactide monomer, commonly by using a stannous octoate based catalyst [1], [5-8].

Firstly, the main uses of PLA have been restricted to medical applications such as tissue scaffolds, implant devices, internal sutures, and others, due to polymer low availability, high cost, and limited molecular weight. Recently, new techniques that allow the economical production of high molecular weight PLA have conclusively led to its larger application, and PLA has taken great attention as an alternative to petrochemical-based synthetic polymers such as polypropylene (PP), poly(ethylene terephthalate) (PET), polycarbonate (PC), and polystyrene (PS), in packaging and/or textile sectors [2], [4].

Despite of its availability and enhanced properties, PLA still has various drawbacks that restrict its utilization in some fields. For instance, although the toughness of PLA is bigger than that of PS, it is still lower than those of PC and PET; this may limit the applicability of PLA as a structural material. In this respect, several methods, involving copolymerization, plasticization, and blending have been applied to overcome the limitations of PLA materials [9-11]. Another disadvantage of PLA is

that it is chemically inert because there are no reactive side chain groups that make their surface and bulk modifications a major challenge. Copolymerization of cyclic lactide monomer with monomers bearing functional groups is the most direct and effective route to introduce reactive groups [12-15].

Infections by pathogenic microorganisms are of great concern in a variety of areas, especially in medical devices, drugs, hospital surfaces/furniture, dental restoration, surgery equipment, healthcare products and hygienic applications such as food packaging, water purification systems, and textiles. Around the world, infectious diseases kill more people than any other single cause and rapid development of antibiotic resistance further makes the situation more serious. However, the use of low molecular weight antimicrobial agents have many disadvantages, such as short-term antimicrobial ability and toxicity to the environment [16-18]. Antimicrobial polymers give promise to deal with such problems by enhancing the efficacy of some existing antimicrobial agents and reducing the environmental problems. In addition, the use of antimicrobial polymers minimizes the residual toxicity of the agents, increases their effectiveness and selectivity, and extends the lifetime of the antimicrobial agents [17-22]. According to their mode of action, these polymers can be divided into two main categories as nonreleasing (contact active) and releasing biocidal action [21]. The contact active (or contact killing) action is usually based upon quaternary ammonium salts that are covalently attached into a polymeric chain or polymeric substrate [23]. However, the released-based biocidal action is generally obtained through the use of biocidal agents such as metallic/inorganic nanoparticles, antibiotics, or others capable of leaching from the polymeric surfaces or materials [22], [23] .

PLA based antimicrobial films are extensively used for food packaging and various types of synthetic and natural antimicrobial agents are impregnated into PLA through solvent casting, melt blending, as well as their combinations. So, it can be said clearly that most of the current studies are related to blending non-volatile antimicrobial agents such as bacteriocin, metals ,enzymes, in addition to chelating agents [24]. However, blending of active agents within a polymer matrix may affect the mechanical, thermal, optical and barrier properties of the packaging material [25], [26]. Hence, there has been increasing interest in the binding of functional

compounds by covalent immobilization onto the food contact surface of packaging films. The most stable linkage between substrate film surface and active agents is provided by covalent bond and the active agents may not need to be defined as food additives, as they are not likely to migrate from the package to the food [25].

In this thesis, the main objective was to prepare antimicrobial PLA based films, to which antimicrobial agents were attached covalently. For this purpose, firstly a six-membered cyclic carbonate monomer with two pendant azido groups, 2,2-bis(azidomethyl)trimethylene carbonate (ADTC) was synthesized [27], [28]. The basic reason for choosing this monomer is that it is easily synthesizable and polymerizable. Also, aliphatic polycarbonates are convenient for our purpose because they are biocompatible, biodegradable and non-toxic materials [29]. Then, this monomer was copolymerized with L-lactide in different molar ratios to obtain a PLA bearing azido-functional group. So, click chemistry was easily carried out between the azido functionalized copolymer and alkynyl quaternary ammonium salts by using Baker's method [30]. The antimicrobial activity tests of these synthesized polymers were carried out against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria by the drop plate method. It was observed that the polymers exhibited highly effective antimicrobial activity. In the second step, the synthesized polymer containing the largest amount of antimicrobial agent was employed to coat one side of neat PLA film, which was obtained by using solvent-casting method, in three different proportions. The antimicrobial activity of obtained films was investigated against *S. Aureus* via contact-active method. And, the films exhibited highly effective antimicrobial activity, even at the lowest coating rate. Moreover, coating process did not alter the thermal, mechanical and barrier properties of the films dramatically. To the best of our knowledge, PLA polymers having antimicrobial activity have been obtained by blending of biocidal agents. In this study antimicrobial agents were attached to PLA via covalent bonds. In addition, the method applied to obtain PLA based antimicrobial surfaces has not been employed before in literature, and this method met our need in obtaining the film with the feature we wanted.

2. THEORETICAL PART

2.1. Biodegradable Polymers

Synthetic plastics are one of the most important commodity considering their widespread usage in all aspects of life including electronics, food packaging, textiles, transportation, healthcare products, toys, building materials, aerospace materials and many other goods [31]. Currently, almost all the used plastics are produced from non-renewable fossil resources and the overuse of these petroleum based plastics is playing a big role in the increase of CO₂ emission into the atmosphere, which is thought to be among the principal reasons for global warming and climate change [32], [33]. As a result, there is an environmental conscience and demand for replacing petroleum derived raw materials with eco-friendly plastics, namely *bio-based plastics* which are produced from renewable resources, or with *biodegradable plastics*, which are suitable for recycling and biodegradation, to solve global environmental and waste management problems [6], [34-36]. Thus, in the last decades both the academic and industrial worlds have given particular importance to bioplastics.

Bioplastics consist of three main categories, on the basis of their origin and biodegradable nature: i) biobased-non-biodegradable bioplastics (e.g., polyethylene terephthalate (PET), polyamide (PA)); ii) bio-based biodegradable bioplastics (e.g., polylactide (PLA), polysaccharides, polyamides, starch); iii) fossil-based biodegradable bioplastics (e.g. poly(vinyl alcohol), polycaprolactone (PCL)) (Figure 2.1) [37], [38]. The terms bio-based polymers and biodegradable polymers are sometimes used alternately, but this is wrong. Biodegradable plastics have been developed from the point of view of biodegradability, whereas bio-based plastics are produced from biomass sources, which are not necessary to be biodegradable [33], [39], [40]. The biodegradability depends on the chemical nature not the origin and biodegradable plastics can be either made of renewable or fossil sources. For example, 100% bio-derived polyethylene terephthalate (PET) and partially bio-derived poly (trimethylene terephthalate) (PTT) are not biodegradable where as 100% petro-derived poly(butyleneadipate-co-terephthalate) (PBAT) is biodegradable [6], [41]. Biodegradable polymers are defined as materials whose physical and

chemical properties undergo degradation when exposed to bacteria, other microorganisms, for example fungal spores to yield carbon dioxide, water, inorganic compounds and biomass [42], [43], [44]. These polymers are classified into four groups according to their origin and synthesis method (Figure 2.2) [37], [45]. First group of biodegradable polymers is directly extracted or removed from renewable polymers. They can be called as agro-polymers. Polysaccharides (cellulose, starch, algin, chitosan), proteins (collagen, gelatin, albumin) and lipids are the example of that group. In the second group of biodegradable polymers, polymers are produced from natural or genetically modified organisms. Polyhydroxyalconoates and poly-3-hydroxybutyrate are can be given as example. The third group of the polymers are produced by polycondensation or ring-opening polymerization of biologically derived monomers. Polyesters such as PLA is a good example for this group. The monomers are produced by the fermentation of sugars. The last group of biogradable polymers is petroleum-based bioplastics. Polycaprolactone (PCL) and polybutylene succinate (PBS) are included in this category.

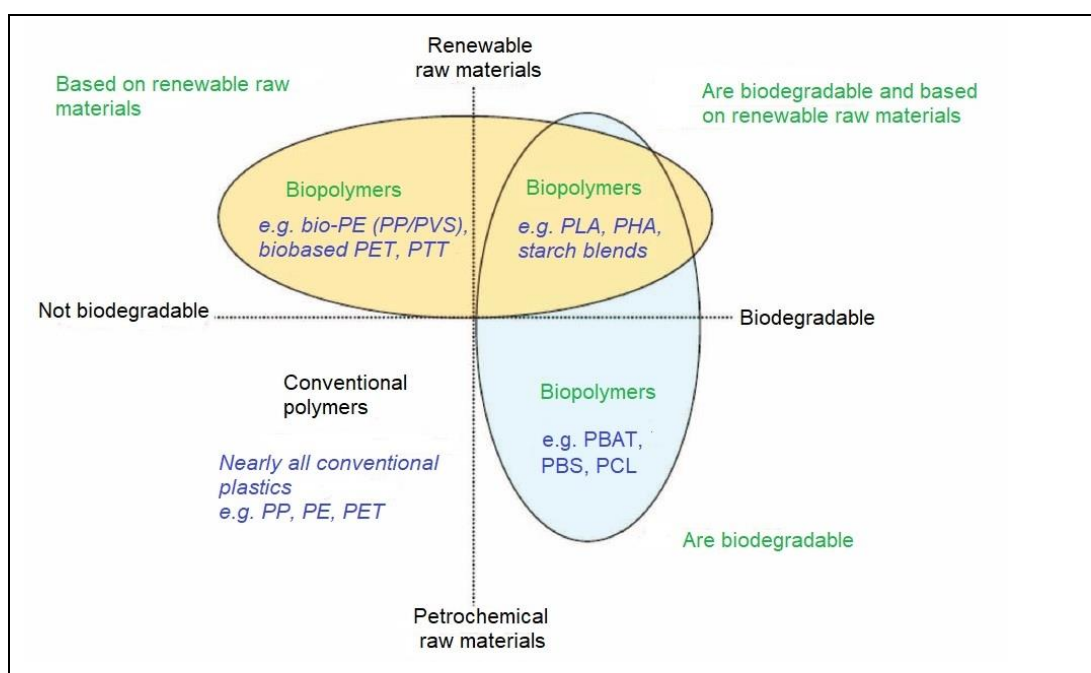


Figure 2.1: Types of bioplastics, both biodegradable and nonbiodegradable, and examples.

The application of biodegradable polymers started several decades ago, and since then much research has been focused on. Biodegradable polymers are

particularly widely used in three main fields: medicine, agriculture and packaging. In medicine, applications of biodegradable polymers include controlled drug delivery and wound management (e.g., sutures, adhesives, and surgical dressings), orthopaedic devices (screws, stents and pins), and for dental repairs and tissue engineering. Because these polymers break down and are absorbed by the body, removal step can now be avoided [46], [47]. Agriculture brings about a significant amount of waste from plastics, estimated at 10% of the total plastic weight in landfills [48]. Thus, the biodegradability and utilization of biodegradable agricultural materials is a topic rising in importance in the last years to reduce the environmental impact of cultivated land.

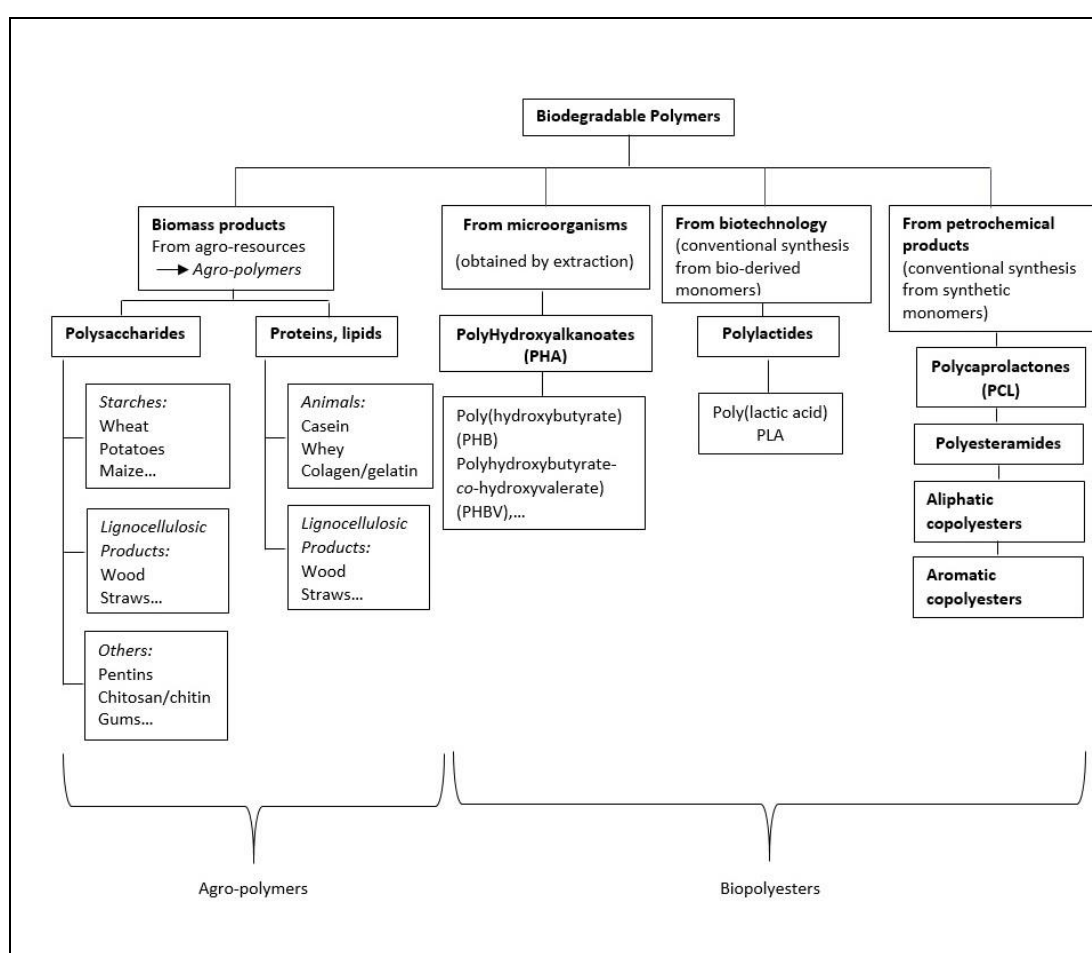


Figure 2.2: Classification of biodegradable polymers.

In addition to agricultural and medical uses, biodegradable polymers are also used in eco-friendly food packaging applications. In order to reduce the volume of waste, the use of biodegradable polymers is quite important. The production

capacities of biodegradable polymers such as polyhydroxylalkanoates (PHAs), poly(lactic acid) (PLA) and starch blends are growing steadily. Among them, polylactide has great commercial potential because of higher thermal and mechanical properties that make it competitive with commodity petroleum based synthetic polymers [32], [49].

2.1.1. What Is Poly(Lactic Acid)?

Poly(lactic acid) (PLA) is a biodegradable and bio-based thermoplastic polyester [40], [50]. Both poly(lactic acid) and polylactide with abbreviation of PLA are in fact the same chemical products; only they differ from each other via production method. Lactic acid (LA), also named 2-hydroxy propionic acid, is the basic monomer of PLA and is made by bacterial fermentation of carbohydrates *obtained from non-toxic renewable resources* such as corn, potatoes, sugarcane and other biomass [3]. PLA is the first commodity polymer produced from annually natural resource. Lactic acid, *a PLA constituent unit*, has two optical isomers: L-(+)-lactic acid or (S)-lactic acid and D-(-)-lactic acid or (R)-lactic acid. L-lactic acid is the natural and biologically crucial isomer, but D-form can be manufactured by microorganisms or by racemization. Stereoisomers of lactic acid are shown in Figure 2.3 [3], [4], [7]. Some physical properties of lactic acid is given In Table 2.1 [51].

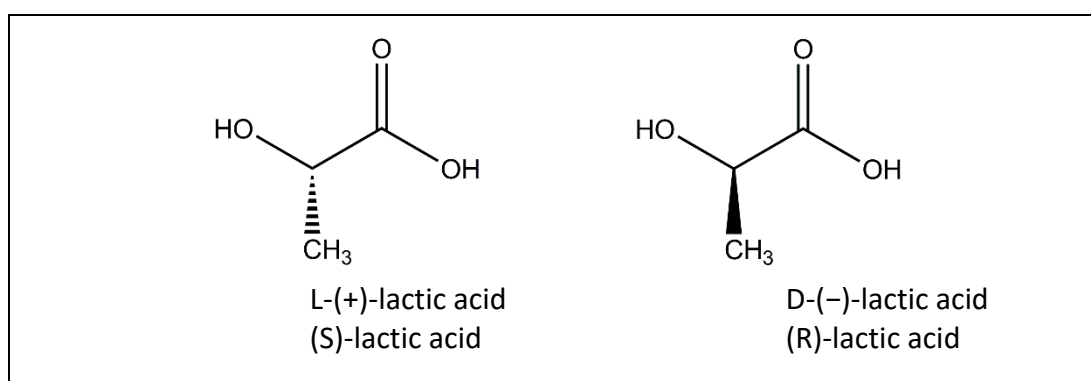


Figure 2.3: Stereoisomers of lactic acid.

Poly(lactic acid) is classified as Generally Recognized as Safe (GRAS) material by the United State Food and Drug Administration (FDA) and is ideal for all food packaging applications [52].

Table 2.1: Physical properties of lactic acid.

Property	Value
CAS number	General: 50-21-5
	(S)-Lactic acid: 79-33-4
	(R)-Lactic acid: 10326-41-7
Molecular weight (g/mol)	90.08
Formula	C ₃ H ₆ O ₃
Melting point (°C)	18 (racemic)
	53 (chiral pure)
Crystal structure	(S)-Lactic acid: orthorhombic, space group $P2_12_12_1$
Solid density (g/mL)	1.33 (solid, 20 °C)
Solubility in water (wt%)	86 (20 °C, monomeric (S)-lactic acid)
Heat of fusion (kJ/mol)	(S)-Lactic acid: 16.8
Boiling Point (°C)	122 (at 14 mmHg)
Liquid density (g/mL, 20°C)	1.224 (100% undercooled liquid)
	1.186 (80.8% solution in water)
Viscosity (mPa s)	28.5 (85.3% solution in water, 25 °C)
pKa	3.86

2.1.2. PLA Production and Application

Today, although there are multiple ways to fabricate PLA, none of them is simple or easy to execute. PLA synthesis requires rigorous control of conditions (temperature, pressure and pH), the use of catalysts and long polymerization times [8]. Generally, the synthesis of polylactides can be prepared by polycondensation reaction of lactic acid or ring-opening polymerization of lactide monomer [40], [53] and Figure 2.4 shows the main methods for PLA synthesis [32], [54]. The major limitation of the polycondensation reaction is to obtain high molecular weight polymer because the viscosity of the mixture during this process tends to increase considerably and the removal of water becomes more difficult, which leads to the

formation of low molecular weight product [7], [11]. Even though the chain extension has been achieved by condensation methods to increase the molecular weight of PLA, the toxicity of chain extenders, as well as the side reactions allied by this method, make the ring-opening polymerization (ROP) of lactide the most acceptable process to obtain an ultrahigh-molecular-weight PLA [8], [11].

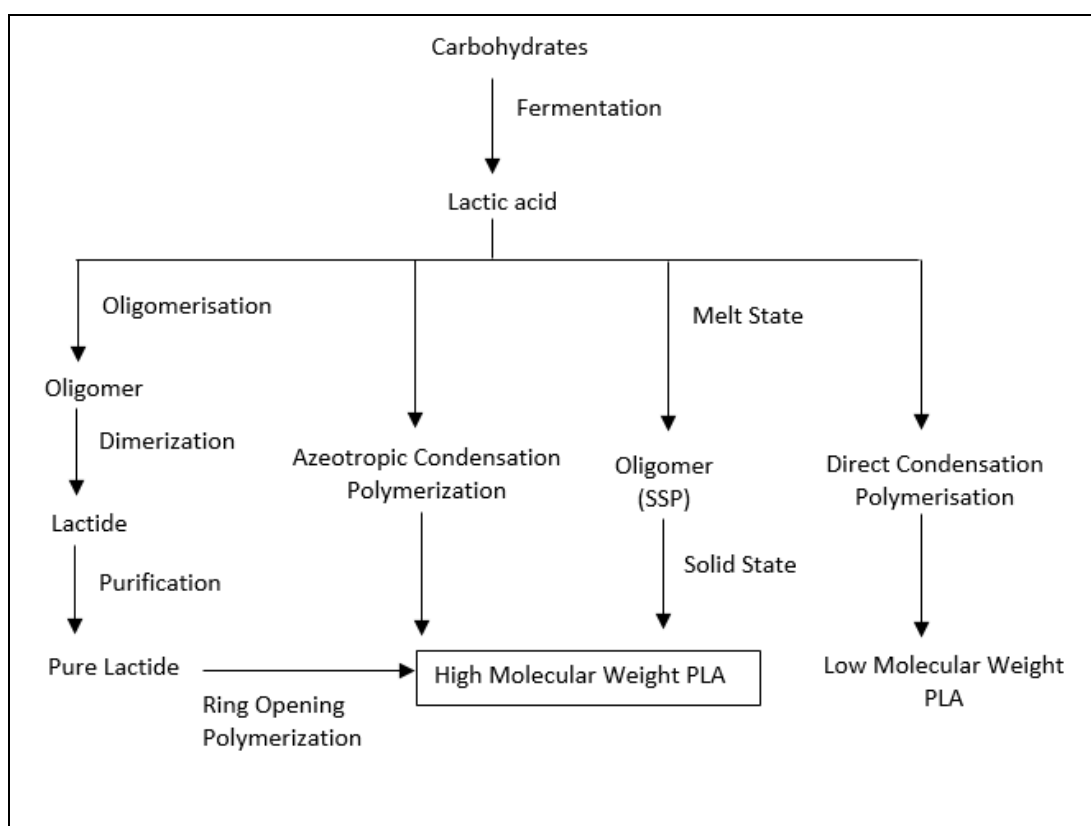


Figure 2.4: Various routes of synthesis of PLA.

Tin(II) bis-(2-ethylhexanoate), also referred to as stannous octoate, tin octoate or $\text{Sn}(\text{Oct})_2$, is the most widely used initiator/catalyst for the synthesis of PLA in relatively short time periods with low racemization and high conversion [55]. This popularity stems from its acceptance by the FDA for food contact, making it ideal for many food packaging applications. Moreover, stannous octoate is less susceptible to water and other protic impurities, which facilitates its use in industry and laboratory [54]. The mechanism of the stannous octoate-mediated ROP of lactones remained a matter of argument for many years, and many different mechanisms were proposed. In 1998, Penzcek and coworkers published kinetic results which strongly suggest that, if polymerization is carried out in THF at 80 °C, then stannous octoate is converted in

situ into a new tin alkoxide by the reaction with either an alcohol, purposely added in the reaction medium, or with any other protic impurity present in the polymerization medium [56]. Tin alkoxides formed in situ are the real initiators of the polymerization, which occur according the usual insertion-coordination mechanism shown in Figure 2.5 [54].

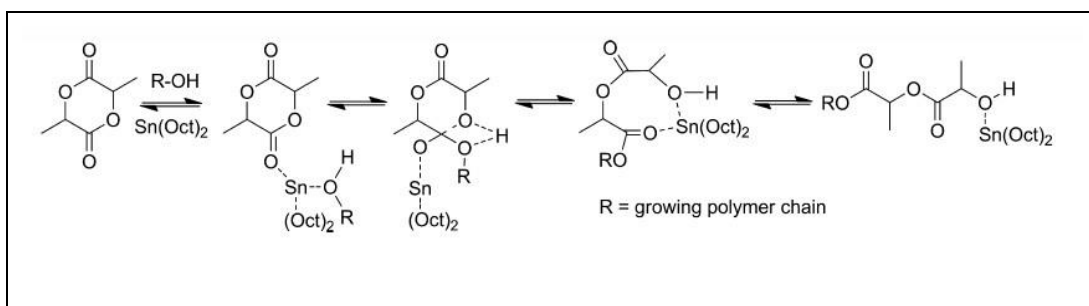


Figure 2.5: Coordination-insertion mechanism of ROP of L-lactide.

2.1.3. Properties and Bulk Modification of Poly(Lactic Acid)

According to other biopolymers, the production of PLA has several advantages [57], including: 1) Eco-friendly — as it derived from natural sources (e.g., corn, wheat, or rice) and, also it is biodegradable, recyclable, and compostable. 2) Biocompatibility — PLA has an important application in the biomedical field because it doesn't produce toxic or carcinogenic effects in local tissues. Besides, its degradation products don't hinder tissue healing. 3) Processibility — PLA can be processed by film extrusion, injection molding, blow molding, fiber spinning, thermoforming and film forming and has better thermal processibility according to other biopolymers such as poly(ϵ -caprolactone) (PCL), poly(hydroxyl alkanooate) (PHA) and poly(ethylene glycol) (PEG). In addition, the mechanical properties such as tensile strength and modulus of PLA are higher than other polyesters (Table 2.2) [58]. 4) Energy savings — less energy 25–55% is required during the production of PLA than other petroleum-based polymers. Lower energy use makes PLA production potentially profitable with respect to cost, too [32]. As a consequence, PLA seems to be the most popular material of choice because its production consumes carbon dioxide. The life cycle of PLA is shown in Figure 2.6 [54].

Table 2.2: PLA mechanical properties compared to those of most common polymers used in commodity applications.

	PLLA	PS	PET	PP	HIPS
Tg (°C)	55-65	105	75	10	-
Tensile strength at break (MPa)	53	45	54	31	23
Tensile modulus (GPa)	3.4	2.9	2.8	0.9	2.1
Elongation at break (%)	6	7	130	120	45
Notched Izod impact strength (J/m)	13	27	59	27 (i-PP)	123
Gardner impact (J)	0.06	0.51	0.32	0.79	11.30

PET, Poly(ethylene terephthalate); PS, Polystyrene; HIPS, High-impact polystyrene; PP, Polypropylene; i-PP, Isotactic polypropylene homopolymer.

Despite its advantages, PLA has some drawbacks that limit its use in applications where mechanical toughness, i.e. plastic deformation at higher stress levels, or high elongation is required (e.g., packaging, appliance casings and car parts, or screws and fracture fixation plates) [57].

Another disadvantage of PLA is that it is chemically inert with no reactive side-chain groups that makes its surface and bulk modifications a big challenge. (Co)polymerization of functional monomers is the most direct and effective route to introduce functionalities [15], [27].

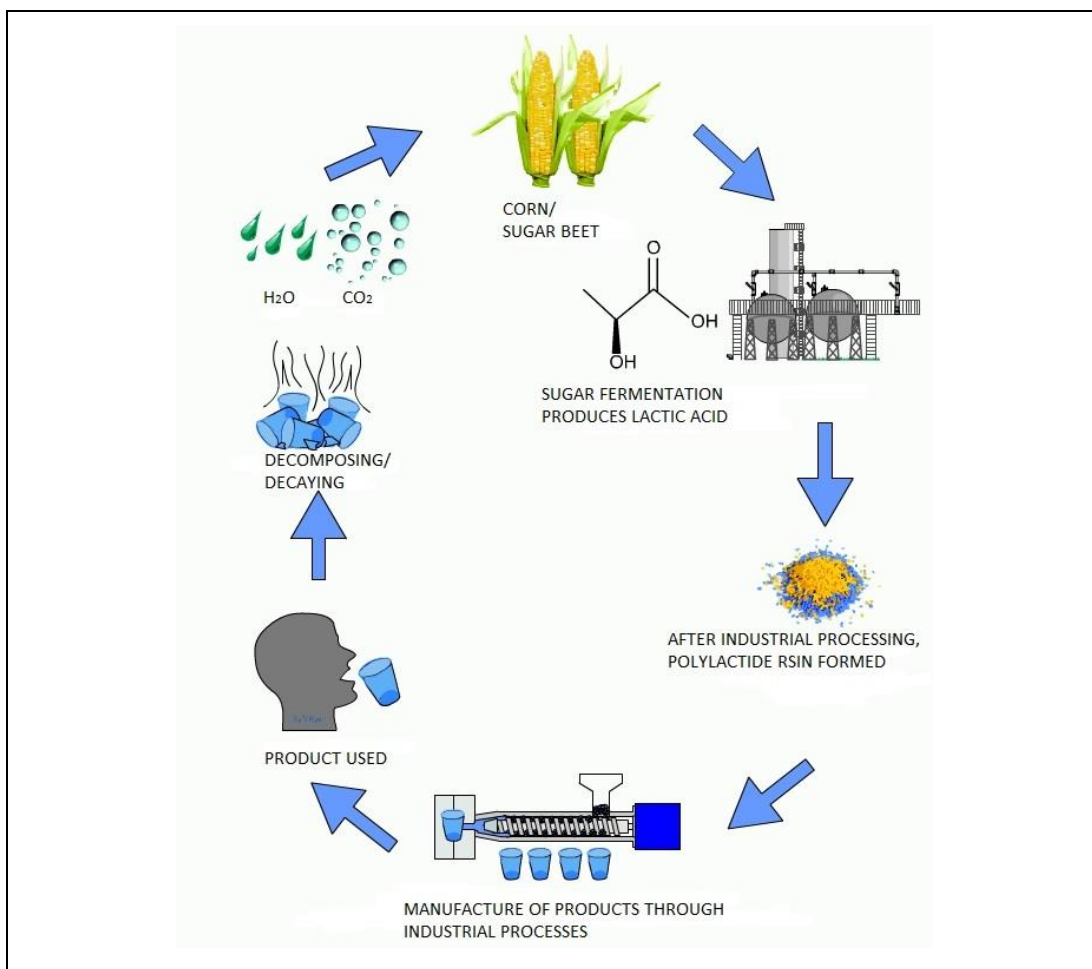


Figure 2.6: Life cycle of PLA.

2.1.3.1 . Effect of Stereochemistry/Crystallinity on Polylactide Properties

The physical, mechanical and thermal properties of PLA and its copolymers are very dependent on the molecular weight and crystallinity and it is possible to control this crystallinity varying the ratio of L and D isomer ratio [59], [60]. Three stereochemical forms can exist: poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and poly(DL-lactide) (PDLLA) depending on L,L-lactide, D,D-lactide and D,L-lactide isomers formed due to the chiral structure of lactic acid (Figure 2.7) [61].

Physical properties are crucial; the dimensions, size, and weight requirements for a desired part or product must be known for a required part or product Also, density is another important design parameter because high density values cause transportation costs (e.g. light car parts reduce energy consumption).

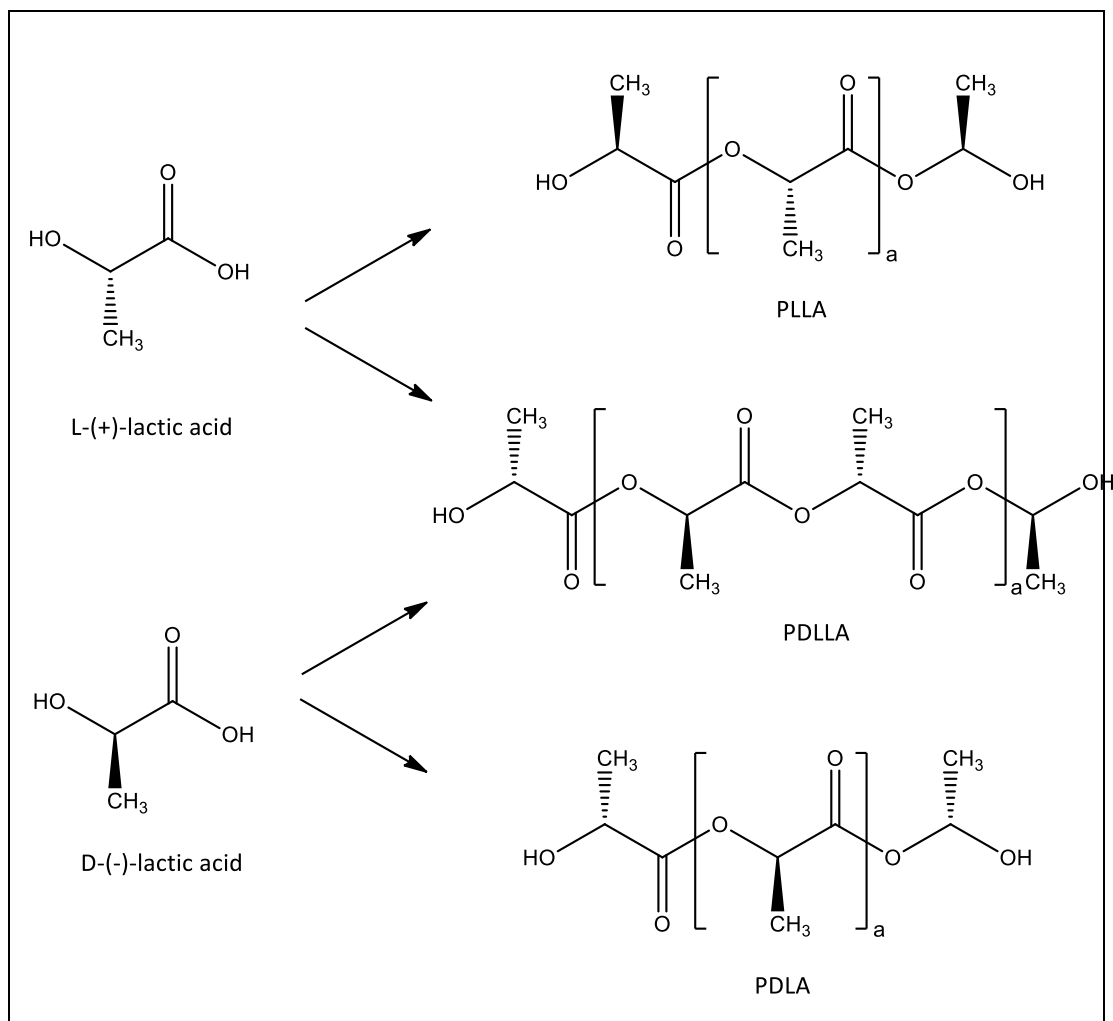


Figure 2.7: Stereo forms of poly(lactic acid). PDLA, poly(D-lactic acid); PDLLA, poly(D-,L-lactic acid); PLLA, poly(L-lactic acid).

The thermal properties of PLA can vary from semicrystalline to amorphous ones by changing the stereochemistry of the polymer (Table 2.3) [39]. It is shown that how the glass transition (T_g) and melting temperature (T_m) of PLA are changed with various ratios of L-monomer composition. The homopolymer of L-lactide (PLLA) is a semicrystalline polymer with a glass transition temperature (T_g) of 55–65°C and a melting point (T_m) of 160–180°C [62]. An amorphous polymer, poly(D,L-lactide) (PLA), with a glass transition temperature, T_g , of around 55–60 °C is obtained when greater than 15% of meso-lactide exists in the monomer feed. Conversely, semi-crystalline polymers can be produced via the direct polymerization of either L-lactide or D-lactide to yield poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA), respectively. These

polymers are obtained with 2 to 20% of D content and have melting temperatures (T_m) around 180 °C [6].

Table 2.3: Variation in glass transition and melting temperature of PLA with various ratios of L-monomer composition.

Copolymer ratio	Glass transition (T_g), °C	Melting temperature (T_m), °C
100:0 (L/DL)-PLA	63	178
95:5 (L/DL)-PLA	59	164
90:10 (L/DL)-PLA	56	150
85:15 (L/DL)-PLA	56	140
80:20 (L/DL)-PLA	56	125

The barrier properties of a polymer is very important to estimate and predict the product-package shelflife. The particular barrier requirement of the package system is connected with the product characteristics and the desired end-use application [41]. Oxygen and water vapour are two of the main permeants examined in packaging applications since they can transfer from the internal or external environment through the polymer package wall, leading a continuous change in product quality and shelf-life. Barrier properties of semi-crystalline polymers are changed according to the crystallinity of the polymer because crystallinity reduces the cross-sectional area for diffusion, increase the diffusion path length and could also impose restriction on the mobility of the amorphous phase. Tsuji et al., showed a correlation among the degree of crystallinity, (X_c), molecular weight and water vapor transmission rate (WVTR) [63].

Barrier properties of PLA particularly to oxygen, carbon dioxide and water vapor have been mostly investigated because it has applications in packaging field. PLA has a better barrier for carbon dioxide, oxygen, and water vapor permeability when compared with PS but a lower barrier when compared with PET as shown Table 2.4 [3]. As diffusion happens through the amorphous regions of a polymer, increasing in the size of crystallization will surely result in reduction in permeability. For

example, poly(98% L-lactide) has higher crystallinity than poly(94% L-lactide) that creates a more complicated path for the permeation of oxygen molecules [60].

Table 2.4: Comparison of water vapor and oxygen transmission rate of PLA and synthetic polymers.

Sample	Thickness (mm)	WVTR [g(m ² /d)]	Permeability Coefficients [kg/m/(m ² /s/Pa)]	OTR [cc(m ² /d)]	Permeability Coefficients [kg/m/(m ² /s/ Pa)]
PET	18	3.48	2.82×10 ⁻¹⁵	9.44	6.95×10 ⁻¹⁹
PS	18	5.18	4.18×10 ⁻¹⁵	531.58	3.91×10 ⁻¹⁷
PLA	20	15.30	1.34×10 ⁻¹⁴	56.33	4.33×10 ⁻¹⁸

The polymer structure plays an important role on the mechanical properties, and consequently on the process utilized to modelling the final product (injection moulding, blow moulding, sheet extrusion, thermoforming, film forming). Tensile test analyses are occurred to determine the elongation at break , tensile strength at break, tensile modulus, and tensile toughness (area under the stress-strain curve). These values are important to obtain mechanical information of the biopolymer materials and to compare with the commercial nonbiodegradable ones (ASTM D882-02, Standard Test Method for Tensile Properties of Thin Plastic Sheeting) [41].

General main goal for PLLA mechanical properties is to increase the tensile toughness and elongation at break without negatively affecting the tensile modulus and tensile strength [10]. For example, PET has a typical strength and modulus of 54 MPa and 2.8 GPa, while polypropylene (PP) exhibits a typical strength and modulus of 31 MPa and 0.9 GPa. However, PLA exhibits a typical tensile strain at break of less than 6%. This brittleness importantly limits PLA's use in many applications [62]. Consequently, researches on polymer modification have gained importance to improve mechanical properties, especially toughness. Several approaches have been developed and copolymerizing lactides with other monomers, plasticization, and blending with a second polymer and composites are toughening routes to increase the elongation of PLA [10], [11], [34] .

Copolymerization-PLA copolymers has been produced either through polycondensation of lactic acid with other monomers, obtaining low molecular weight copolymers, or ring opening copolymerization of lactide with cyclic monomers like ϵ -caprolactone, glycolide, trimethylene carbonate, δ -valerolactone etc. obtaining high molecular weight copolymers. Ring opening copolymerization (ROC) [7] of lactide monomer is a common method for PLA copolymer synthesis, initiated with hydroxyl groups, such as alcohol or polyol. ROC has been used broadly because of its precise chemistry control and resulting agreeable copolymer properties.

Blending with biodegradable/renewable-resource polymer - Blending PLA with biodegradable ductile polymers are often used method to improve toughness without compromising biodegradability. PLA/PCL is broadly investigated biodegradable PLA blend system. PCL is a rubbery polymer with low T_g , and this rubbery PCL with much slower degradation rate exhibits better toughness though PLA with high degradation rate exhibits better tensile strength. The compatibility between these two biodegradable polymers is very important for their blend materials since the performance can be controlled by arranging the blending ratio to apply in various applications [7], [39], [62].

Plasticization-Plasticizers are generally used to enhance the the processability of thermoplastics, in addition to increase the ductility and flexibility of glassy polymers [64]. To be useful, the plasticizer and polymer must mix well and so, lower the glass transition temperature of the polymer [65]. For example, commercial PLA, i.e. poly(92% L-lactide, 8% meso-lactide), has a modulus of 2.1 GPa and an elongation at break of 9%. Its Young's modulus decreases to 0.7 MPa and the elongation at break increases to 200% after plasticization, in accordance with T_g variation from 58 °C to 18 °C [34]. This example demonstrated that mechanical properties can be readily modified to make it suitable for desired applications.

3. ANTIMICROBIAL POLYMERS

Infections by pathogenic microorganisms are of great concern in a variety of areas, especially in medical devices, drugs, hospital surfaces/furniture, dental restoration, surgery equipment, healthcare products and hygienic applications such as food packaging, water purification systems, and textiles. Around the world, infectious diseases kill more people than any other single cause and rapid development of antibiotic resistance further makes the situation more serious. So, it is necessary to maintain these areas from these microorganisms. It is usually possible to kill microorganisms by conventional disinfectants and antibacterial agents of low molecular weight such as hypochlorite, hydrogen peroxide, quaternary ammonium compounds, silver salts or other reactive oxygen species. However, the use of these low molecular weight antimicrobial agents is a daunting challenge due to environmental contamination caused by residues, toxicity to the human body and the short action duration which results in contaminated surfaces again after exposure to the pathogens. Taking these limitations into account, there is a definite need to develop new macromolecules with antimicrobial properties and antimicrobial polymers fulfill this requirement. Antimicrobial polymers can be defined as materials that can kill/inhibit the growth of microbes on a surface or surrounding environment. They offer promise for prolonged lifetime, reduced residual toxicity and enhanced microbicidal activity. In addition, they do not permeate through skin because they are chemically stable and non-volatile [12], [16-18] .

Even though there are several ways to classify these systems, Bonilla et al. classified antimicrobial into four different categories (Figure 3.1) [19]: a) polymers exhibit antimicrobial activity by themselves; b) polymers that biocidal activity is provided via chemical modification; c) polymers that contain antimicrobial organic compounds with either low or high molecular weight (Mw); d) polymers involving antimicrobial inorganic compounds.

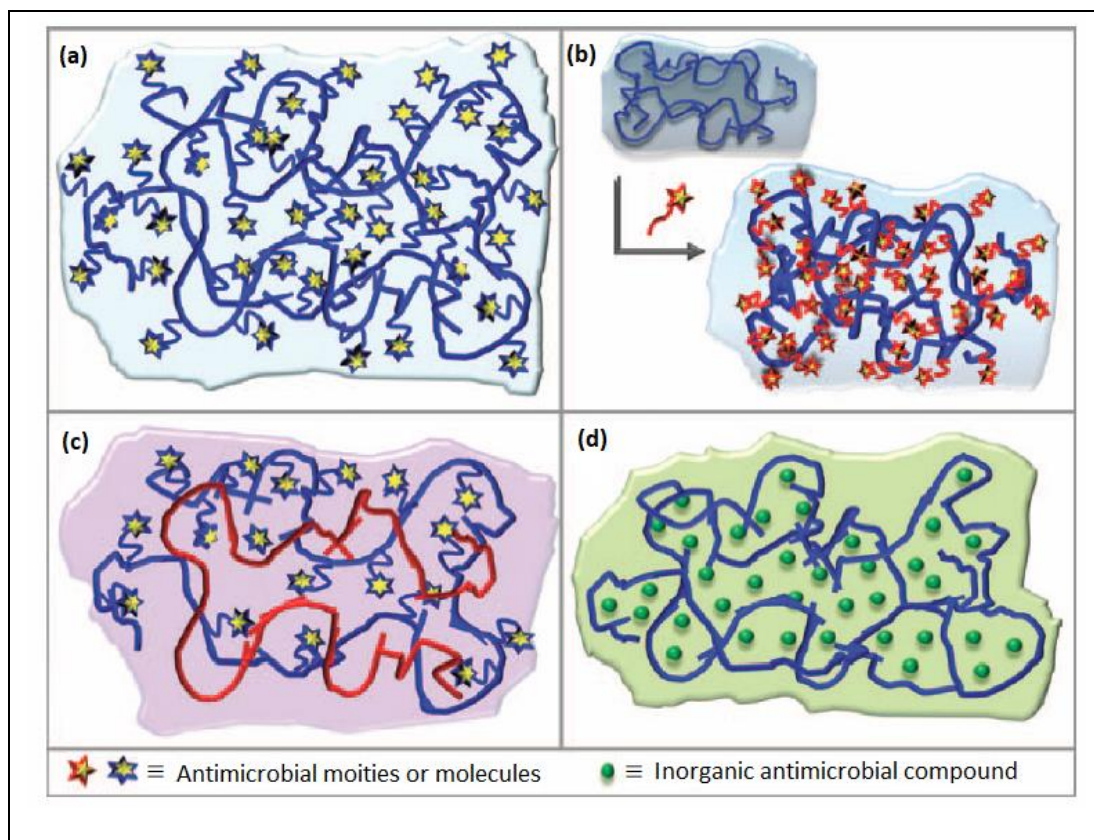


Figure 3.1. Representation of (a) polymers exhibit antimicrobial activity themselves; (b) polymers that biocidal activity is provided via chemical modification; (c) polymers that contain antimicrobial organic compounds (either of low or high molecular weight) with antimicrobial activity into polymers and; (d) polymers involving antimicrobial inorganic compounds to achieve antimicrobial activity.

- i) Polymers exhibit antimicrobial activity by themselves. As their name indicates these are polymers with inherent antimicrobial activity. Therefore, polymers with quaternary nitrogen atoms in their backbone, chitosan, poly- ϵ -lysine, Polyethylenimine, Polyguanidines, and N – halamines can give as example for this category.
- ii) Polymers that biocidal activity is provided via chemical modification. There are different methods to attach antimicrobial activity into polymers. i) a small molecule with antimicrobial activity is covalently incorporated to the polymer; (ii) antimicrobial peptides are attached to an inactive polymer; iii) antimicrobial polymers are grafted to regular polymers. It is required in all cases that chemical modification does not affect negatively the properties of the final polymeric material.

- iii) Polymers that contain antimicrobial organic compounds with either low or high molecular weight (M_w). In this case, the antimicrobial activity is provided via (i) noncovalent bonds between antimicrobial agents, either natural or synthetic, and polymers with the corresponding compound releasing and (ii) the mixture/blend of antimicrobial polymers and nonactive polymers to attain their biocide characteristics.
- iv) Polymers involving antimicrobial inorganic compounds. In this category, the antimicrobial activity in the final material is achieved by transfer of the biocidal inorganic systems, such as metals, metallic oxides or modified clays, into the polymers.

According to their mode of action, these polymers can be also divided into two main categories as nonreleasing (contact active) and releasing biocidal action (Figure 3.2) [21]. In Figure 3.1, the polymers in categories (a) and (b) are nonreleasing (contact-active) polymers [66], [67], while the other two are releasing systems [68], [69]. The contact active (or contact killing) action is usually based upon quaternary ammonium salts that are covalently attached into a polymeric chain or polymeric substrate. However, the released-based biocidal action is generally obtained through the use of biocidal agents such as metallic/inorganic nanoparticles, antibiotics, or others capable of leaching from the polymeric surfaces or materials [23].

As aforementioned, the leaching of active agents causes limited service time for activity and release of these low molecular weight antibacterial agents to the environments can cause human toxicity and to somehow bacteria resistance. Preparation of polymeric antimicrobial coatings via covalently attached biocides to provide the contact killing ability is a growing field in the area of hygiene surfaces.

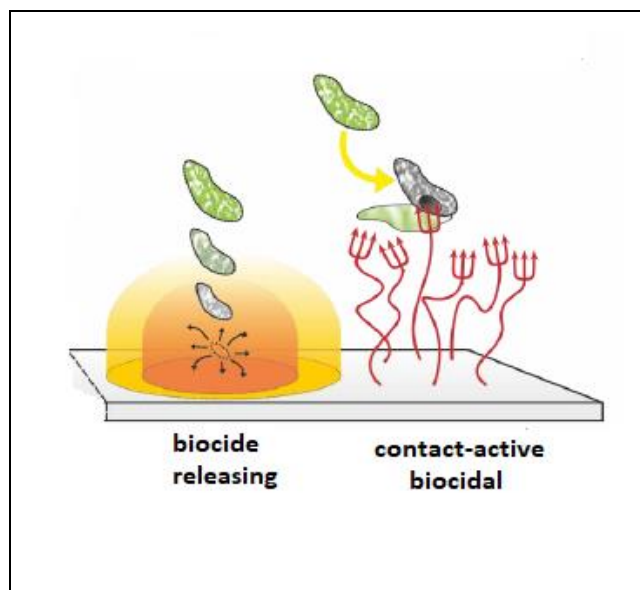


Figure 3.2: General principles of antimicrobial surfaces according to mode of action.

This approach provides several advantages, involving preventing the release of biocides into the environment, prolonged lifetime of coating and increased antibacterial selectivity / efficiency in the case of high local concentration of biocides at the surface of materials [69].

3.1. Mechanisms of Action of Cationic Polymers against Micro-Organism

Developments in the methods of examination of macromolecules such as atomic force microscopy (AFM), dynamic light scattering (DLS), transmission electron microscopy (TEM), fluorescence microscopy, calorimetry (differential scanning calorimetry, isothermal titration calorimetry) etc. have contributed to the mechanism of antimicrobial polymers interactions between bacterial cells and model phospholipid membranes [20]. Positively charged cationic polymers bind to negatively charged cell wall membrane of bacteria easily, diffuse through cell wall and bind onto the cytoplasmic membrane. After the cytoplasmic membrane is disrupted, K^+ ion and other cytoplasmic constituents release and the cell death occurs [70], [71].

3.2. PLA Based Antimicrobial Films For Food Packaging

Antimicrobial packaging can be defined as food-packaging systems that hinder spoilage and decrease pathogenic microorganisms. The packaging having antimicrobial property helps to prolong the shelf life of foods by extending the lag period of microorganisms, thereby decreasing their growth and number [28, 126]. Antimicrobial packaging is aimed to act against microorganisms and develop the functions of conventional food packaging, that are (1) shelf life extension, (2) maintenance of quality, and (3) safety assurance [26]. For instance, the contamination of refrigerated foods, which occur predominantly on the surface of the foods, can be avoided by the application of antimicrobial packaging materials [72]. Food-packaging films with antimicrobial activity can be categorized into two groups: (1) films that permit the antimicrobial to diffuse into the food (2) films that do not migrate their antimicrobial substances and that prevent microbial growth on the food surface [26], [73]. In the first case, a antimicrobial agent is found either within the matrix or on the surface of the food-packaging material. The corresponding agent can be released exactly or in a specific amount on the food surface to practice its biocide action. A schematic representation is shown for the first case in Figure 3.3 [26], in which (A) exemplifies a packaging system that includes the antimicrobial agent in a single layer and releases it into the food matrix gradually, (B) presents the same approach but with an inner layer, that can be useful to control the release of the antimicrobial compound, and (C) forms from a layer of food-packaging material coated with a formulation including an antimicrobial substance [74], [75]. In the second case, the scheme (D) in Figure 3.3 demonstrates a packaging system in which antimicrobial activity takes place only when microorganisms get in touch with the surface of the packaging material. For both types of systems, direct contact with the food is mandatory, making these technologies an appropriate option for vacuum-packed foods such as cheese, fish, meat, or poultry [76].

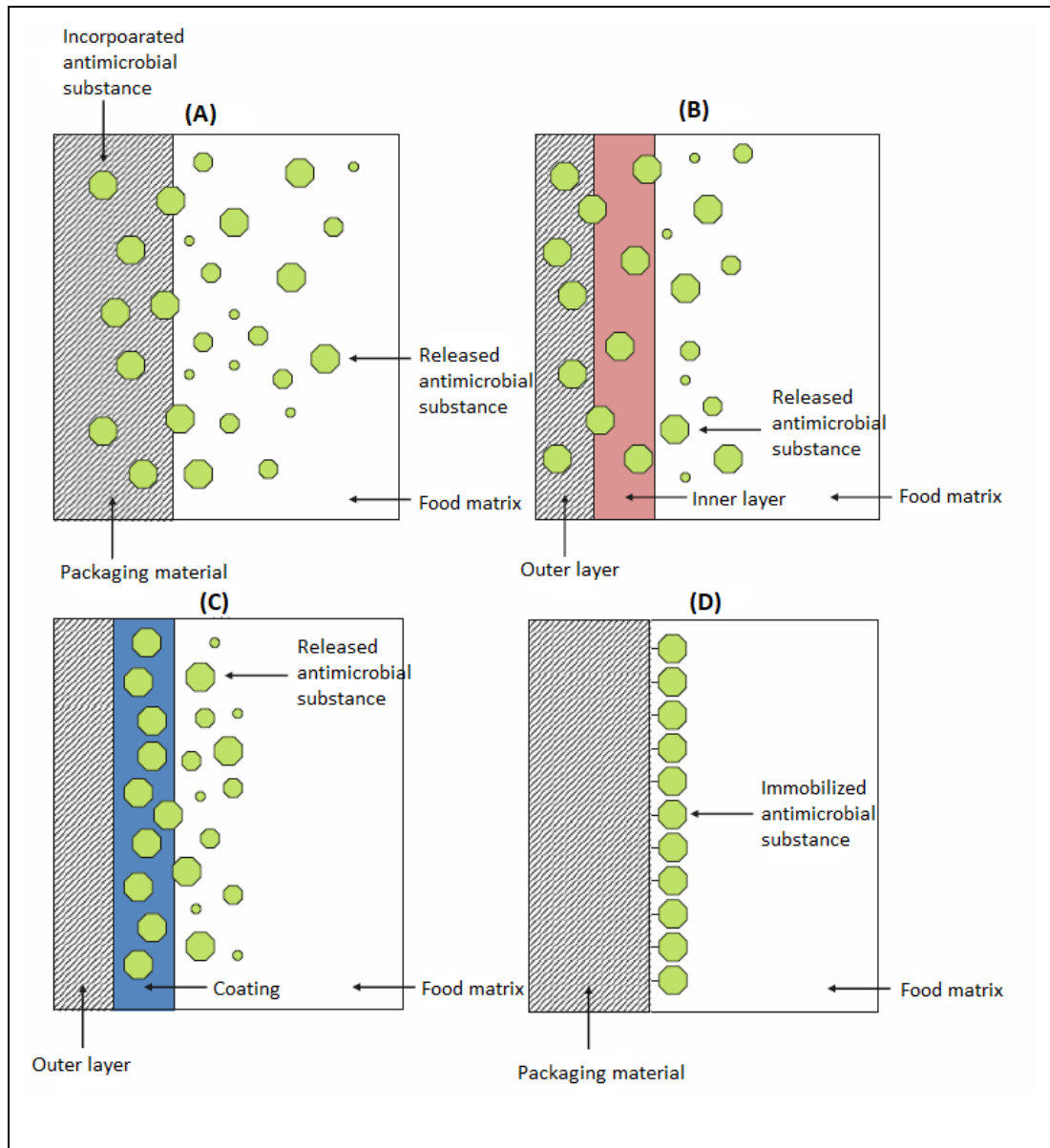


Figure 3.3: Antimicrobial food-packaging systems.

3.2.1. Engineering Properties of Antimicrobial Food-Packaging Films

Mechanical and physical properties of many packaging materials may change after the incorporation of antimicrobial agents. The antimicrobial agent can be embodied with minimal physico-mechanical property deterioration when it is compatible with the packaging material. However, an excess amount of an incompatible antimicrobial agent may considerably affect the physico-mechanical properties of the obtained composite material. Bastarrachea and others [26] have reviewed the engineering characteristics of several polymeric antimicrobial materials

for food packaging. They stated that a few changes happened in the mechanical, thermal, and gas barrier properties of the polymer, as in the surface morphology as a consequence of the addition of the antimicrobial agent. Usually, the mechanical properties of antimicrobial coated polymeric materials are not expected to be changed significantly by the antimicrobial coating. In such cases, the polymeric material is a supporting layer for the coating. But, some interaction between the polymer and coating system may take place at the interface, especially when the antimicrobial agent concentration is high. Jin and others [77] have obtained an antimicrobial film involving PLA and pectin with nisin loaded into the film at 1% (w/w) concentration via a diffusion coating method. Even though they indicated that the coating of nisin did not alter the overall properties of the PLA/pectin film, important changes in the mechanical properties were occurred with the addition of nearly 20% (w/w) pectin and 6.7% (w/w) water into the PLA films.

There are a couple of studies that have analyzed the thermal properties of antimicrobial PLA packaging films. This may be because of the small or unimportant changes in the thermal profile by using thermogravimetric analysis (TGA) of the PLA films integrated with antimicrobial agents [24]. The entrapment of low concentrations of antimicrobial agents at levels less than 10% (w/w) for instance, will generally have only a very slight effect on the T_g , melting temperature (T_m), crystallinity temperature (T_c) or the percentage of crystallinity (X_c). Another example, Liu and others [78] examined the thermal properties of PLA blended with 5% (w/w) nisin and etilendiamin tetraasetik asit (EDTA) that was plasticized by using 30% (w/w) glycerol triacetate (GTA).

4. MATERIAL AND METHOD

4.1. Synthesis of Quaternized Ammonium Functionalized PLA

The chemicals and their properties used in this study are listed in Table 4.1.

Table 4.1: The chemicals used in antimicrobial polymer synthesis, separation and purification processes.

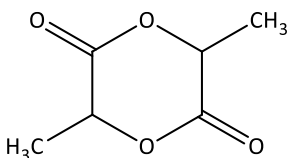
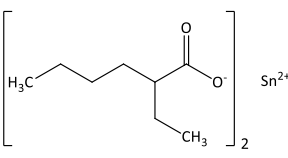
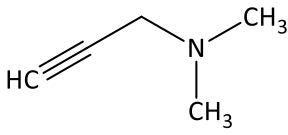
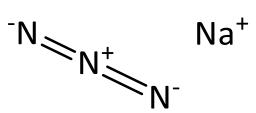
Name	Company	CAS Number and Chemical Structure	Properties ^{[79], [80]}
(3S)-cis-3,6-dimethyl-1,4-dioxane-2,5-dione, 98% (L-Lactide)	Sigma-Aldrich	4511-42-6 	M: 144.12 g·mol ⁻¹ mp: 92-94 °C p: 1,33 g mL ⁻¹ at 20 °C Solubility: 167 mg/L for toluene
Tin(II) ethylhexanoate (Sn(Oct) ₂), 95%	Sigma-Aldrich	301-10-0 	M: 405.122 g·mol ⁻¹ p: 1.251 g mL ⁻¹ mp: < 0 °C Apperance: Yellow liquid Solubility in water: Degrades in water to form Sn(IV)
3-Dimethylamino-1-propyne, 97% (N,N-dimethylprop-2-yn-1-amine)	Sigma-Aldrich	7223-38-3 	M: 83.13 g·mol ⁻¹ p: 0.772 g mL ⁻¹ at 25 °C mp: 18°C (estimate) bp: 79-83 °C Apperance: Clear colorless to yellow Solubility in water: Fully miscible
Sodium azide (NaN ₃), 99.5%	Sigma-Aldrich	26628-22-8 	M: 65.0099 g·mol ⁻¹ p: 1.846 g mL ⁻¹ at 20 °C mp: 275 °C Apperance: colorless to white

Table 4.1: Table of Continuation.

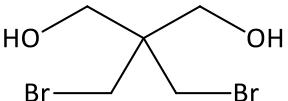
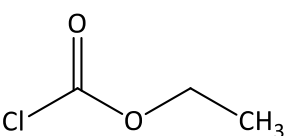
2,2-Bis(bromomethyl)-1,3-propanediol, 98%	Sigma-Aldrich	3296-90-0 	M: 261,94 g·mol ⁻¹ ρ: 2.2 g mL ⁻¹ at 20 °C mp: 112-114 °C bp: 107 – 109 °C Apperance: Off-white powder. Solubility in water: Slightly soluble.
Ethyl chloroformate, 97%	Sigma-Aldrich	541-41-3 	M: 108,52 g·mol ⁻¹ ρ: 1.1403 g mL ⁻¹ mp: -81 °C bp: 93 °C Apperance: Clear liquid Solubility: water: insoluble alcohol: miscible benzene: miscible
1-bromododecane, 98%	Alfa Aesar	143-15-7 $\text{CH}_3(\text{CH}_2)_9\text{CH}_2\text{Br}$	M: 249.23 g·mol ⁻¹ ρ: 1.038 g mL ⁻¹ at 25 °C mp: -11--9 °C bp: 134-135 °C/6 mmHg
1-bromohexane, 98%	Alfa Aesar	111-25-1 $\text{CH}_3(\text{CH}_2)_4\text{CH}_2\text{Br}$	M: 165.074 g·mol ⁻¹ ρ: 1.176 g/mL mp: -84.70 °C bp: 154 to 158 °C Apperance: Colorless liquid Solubility in water: Insoluble Solubility: Alcohol, ether
1-bromopropane, 99%	Alfa Aesar	106-94-5 $\text{CH}_3\text{CH}_2\text{CH}_2\text{Br}$	M: 122.993 g·mol ⁻¹ ρ: 1.354 g mL ⁻¹ mp: -110.5 °C bp: 70.3 to 71.3 °C Apperance: Colorless liquid Solubility in water: 2.5 g L ⁻¹ (at 20 °C)

Table 4.1: Table of Continuation.

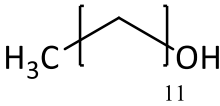
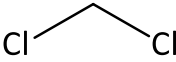
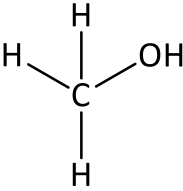
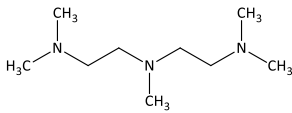
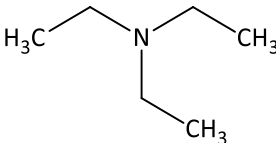
1-Dodecanol, 98%	Sigma-Aldrich	112-53-8 	M: 186,34 g·mol⁻¹ ρ: 0.831 g mL⁻¹ mp: 24 °C bp: 259 °C Apperance: Colorless liquid Solubility in water: 0.004 g/L Solubility: Ethanol, diethyl ether
Dichloromethane (DCM)	Sigma-Aldrich	75-09-2 	M: 84.93 g·mol⁻¹ ρ: 1.3266 g mL⁻¹ mp: -96.7 °C bp: 39.6 °C Apperance: Colorless liquid Solubility in water: 17.5 g/L (25 °C)
Methanol	Sigma-Aldrich	67-56-1 	M: 32.04 g mol⁻¹ ρ: 0.792 g mL⁻¹ mp: -97.6 °C bp: 64.7 °C Apperance: Colorless liquid Solubility in water: Miscible
<i>N,N,N',N'',N'''</i> -Pentamethyldiethylenetriamine (PMDETA), 99%	Aldrich	3030-47-5 	M: 173.304 g·mol⁻¹ ρ: 0.830 g mL⁻¹ mp: -20 °C bp: 198 °C
Triethylamine (TEA) ≥ 99.5%	Sigma-Aldrich	121-44-8 	M: 101.193 g·mol⁻¹ ρ: 0.7255 g mL⁻¹ mp: -114.70 °C bp: 88.6 to 89.8 °C

Table 4.1: Table of Continuation.

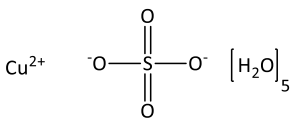
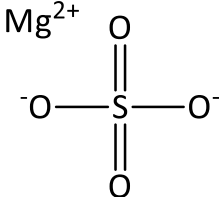
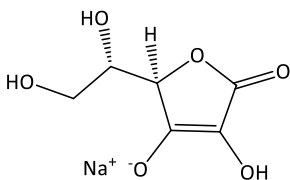
Copper(II)sulfate pentahydrate, $\geq 98\%$	Sigma-Aldrich	7758-99-8 	M: 249.685 g·mol⁻¹ p: 2.286 g mL⁻¹ mp: 110 °C (230 °F; 383 K) decomposes (·5H₂O) bp: decomposes Apperance: blue Solubility in water: 1.26 molal (20 °C) Solubility: soluble in methanol 10.4 g/L (18 °C). Insoluble in ethanol.
Magnesium sulfate (MgSO ₄)	Sigma-Aldrich	7487-88-9 	M: 120.366 g mol⁻¹ (anhydrous) mp: anhydrous decomposes at 1,124°C Apperance: white crystalline solid Solubility in water: Anhydrous 35.1 g/100 mL (20 °C) Solubility: 1.16 g/100 mL (18°C, ether)
Sodium ascorbate, $\geq 98\%$	Sigma	134-03-2 	M: 198.106 g·mol⁻¹ p: 1.66 g mL⁻¹ mp: 218 °C (decomposes) Apperance: minute white to yellow crystals Solubility in water: 62 g/100 mL (25 °C) Solubility: very slightly soluble in alcohol. Insoluble in chloroform, ether.

Table 4.1: Table of Continuation.

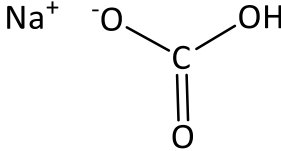
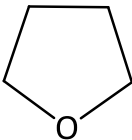
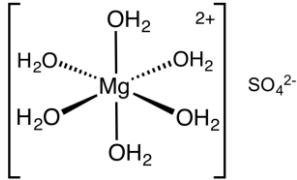
Sodium bicarbonate (NaHCO ₃), ≥ 99.7%	Sigma-Aldrich	144-55-8 	M: 84.0066 g mol ⁻¹ p: 2.20 g mL ⁻¹ mp: Decomposes to sodium carbonate starting at 50 °C. Apperance: White crystals Solubility in water: 96 g/L (20 °C) Solubility: 0.02 wt% acetone, 2.13 wt% methanol at 22 °C.
Sodium chloride, 99%	Alfa-Aesar	7647-14-5 	M: 58.443 g mol ⁻¹ p: 2.17 g mL ⁻¹ mp: 800.7 °C bp: 1,465 °C Apperance: Colorless cubic crystals Solubility in water: 360 g/L
Tetrahydrofuran, anhydrous, ≥99.9%	Sigma-Aldrich	109-99-9 	M: 72.107 g·mol ⁻¹ p: 0.8876 g mL ⁻¹ at 20 °C mp: -108.4 °C bp: 66 °C Apperance: Colorless liquid Solubility in water: Miscible
Magnesium sulfate (anhydrous), (MgSO ₄), 99.5%	Sigma-Aldrich	7487-88-9 	M: 120.366 g·mol ⁻¹ p: 2.66 g mL ⁻¹ mp: anhydrous decomposes at 1,124°C Apperance: White crystalline solid Solubility in water: Anhydrous 35.1 g/100 mL (20 °C) Solubility: slightly soluble in alcohol, glycerol.

Table 4.1: Table of Continuation.

Calcium hydride, 95%	Sigma-Aldrich		7789-78-8 H^- Ca^{+2} H^-	M: 42.094 $\text{g}\cdot\text{mol}^{-1}$ ρ: 1.70 g mL^{-1} , solid mp: 816 °C Apperance: gray powder (white when pure)
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The devices used in this study are listed in Table 4.2.

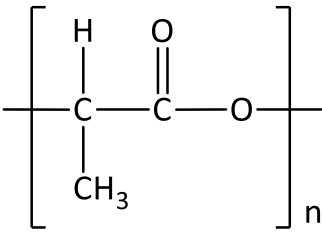
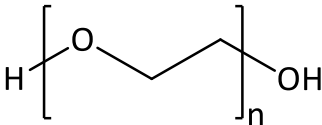
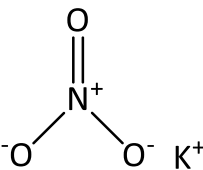
Table 4.2: The devices used in the characterization studies.

Name	Condition
Differential Scanning Calorimetry (DSC)	DSC 4000 (Perkin Elmer) instrument under a nitrogen flow of 10 mL/min. The samples were first heated from 25 °C to 150 °C, then cooled to 0 °C, and finally heated to 150 °C at a scan rate of 10 °C min^{-1} .
Thermal Gravimetric Analysis (TGA)	TGA/SDTA 851 (Mettler Toledo) thermogravimetric analyzer from room temperature up to 700 °C at a heating rate of 10 °C min^{-1} under nitrogen atmosphere.
Fourier-transformInfrared Spectroscopy (FTIR)	Perkin Elmer Spectrum Two TM spectrometer equipped with UATR accessory.
Gel Permeation Chromatography (GPC)	Agilent 1260 Infinity GPC/SEC instrument consisting of a pump, a refractive index detector and two Agilent PLgel columns (Mixed-C, 5 μm , 7.5 × 300 mm) at 40 °C which was calibrated with linear polystyrene standards.
Nuclear Magnetic Resonance Spectroscopy (NMR)	Varian ^{UNITY} INOVA 500 spectrometer at 25 °C in CDCl_3 solutions relative to the non-deuterated solvent traces as the internal reference.
Scanning Electron Microscope (SEM) and SEM energy dispersive X-ray (SEM-EDX)	Scanning electron microscopy and energy dispersive X-ray analysis were performed with QUANTA FEG-250 Field Emission Scanning Electron Microscope (FEI Company, USA) with low vacuum detector (LFD).

4.2. Preparation of Antimicrobial PLA Film

The chemicals and their properties used in this study are listed in Table 4.3.

Table 4.3: The chemicals used in antimicrobial PLA film preparation and characterization.

Name	Company	CAS Number and Chemical Structure	Properties
Polylactide resin	PLA 2002D, Natureplast	9051-89-2 	Mn=155,500 and D-isomer = 3.5%, MFI = 2.9 g/10 min (Dubois)
PEG 1000	Aldrich	25322-68-3  Average Number of Repeating Oxyethylene Units, n: 22.3	M_w : 950-1050 ρ : 1.093 g mL ⁻¹ at 60°C mp : 34 – 40 °C Apperance : Waxy solid Solubility in Water : 80 % by weight, at 20°C.
Calcium chloride anhydrous, ≥97%	Sigma Aldrich	10043-52-4 	M : 110.98 g·mol ⁻¹ ρ : 2.15 g mL ⁻¹ mp : 772–775 °C bp : 1,935 °C Solubility in water : 4.5 g/100 mL (20 °C) Solubility : Soluble in acetic acid, alcohols.
Potassium Nitrate	Sigma-Aldrich	7757-79-1 	M : 101.1032 g·mol ⁻¹ ρ : 2.109 g mL ⁻¹ at 16 °C. mp : 334 °C bp : 400 °C (decomposes) Solubility in water : 242 g/L (20 °C)

5. EXPERIMENTAL PART

5.1. Synthesis of Quaternized Ammonium Functionalized PLA

5.1.1. General Procedure

In this part the main objective was to synthesize PLA, to which antimicrobial agents were covalently attached (Figure 5.1). The reactions were performed in inert atmosphere (dry argon atmosphere) in order to prevent chemicals from influence of oxygen and moisture in the air. The glassware used in the reactions were dried in the burner flame, argon was passed through them and used after filling with argon by cooling.

5.1.2. Pre-Preparation for Experiments

Triethylamine (TEA) and dimethylformamide (DMF) were dried over CaH_2 and distilled before use. Tetrahydrofuran (THF) and toluene were purified by distillation with sodium and benzophenone. L-Lactide monomer was recrystallized from dry toluene for three times before use.

5.1.3. Synthesis of 2,2-bis(azidomethyl)propane-1,3-diol (**1**)

2,2-Bis(azidomethyl)propane-1,3-diol (**1**) was synthesized according to the literature procedure [81], [82] with minor modifications. Briefly, 2,2-bis(bromomethyl)propane-1,3-diol (98%, 40.00 g, 153 mmol) was dissolved in 200 mL DMF, and then NaN_3 (39.71 g, 611 mmol) was added to this solution under argon. The reaction mixture was heated to 120 °C and refluxed for 48 h. After being cooled to room temperature, the formed precipitate was filtered off and DMF was evaporated. The residue was diluted with ethyl acetate (100 mL) and transferred to an extraction funnel. This solution was then washed successively with brine (3 × 100 mL) and water (2 × 100 mL). The organic phase was dried with anhydrous MgSO_4 . A yellowish oil (25.53 g, 130.3 mmol, 80% yield) was obtained after removing the volatiles under vacuum.

$^1\text{H NMR}(\text{CDCl}_3, 500\text{MHz})$: δ 3.62 (s, $-\text{CH}_2\text{N}_3$, 4H), 3.41 (s, $-\text{CH}_2\text{OH}$, 4H), 2.35 (br, $-\text{OH}$, 2H). $^{13}\text{C NMR}(\text{CDCl}_3)$ δ takes the values 63.7, 51.8 and 44.9 (see Figure B1.1, Appendix B).

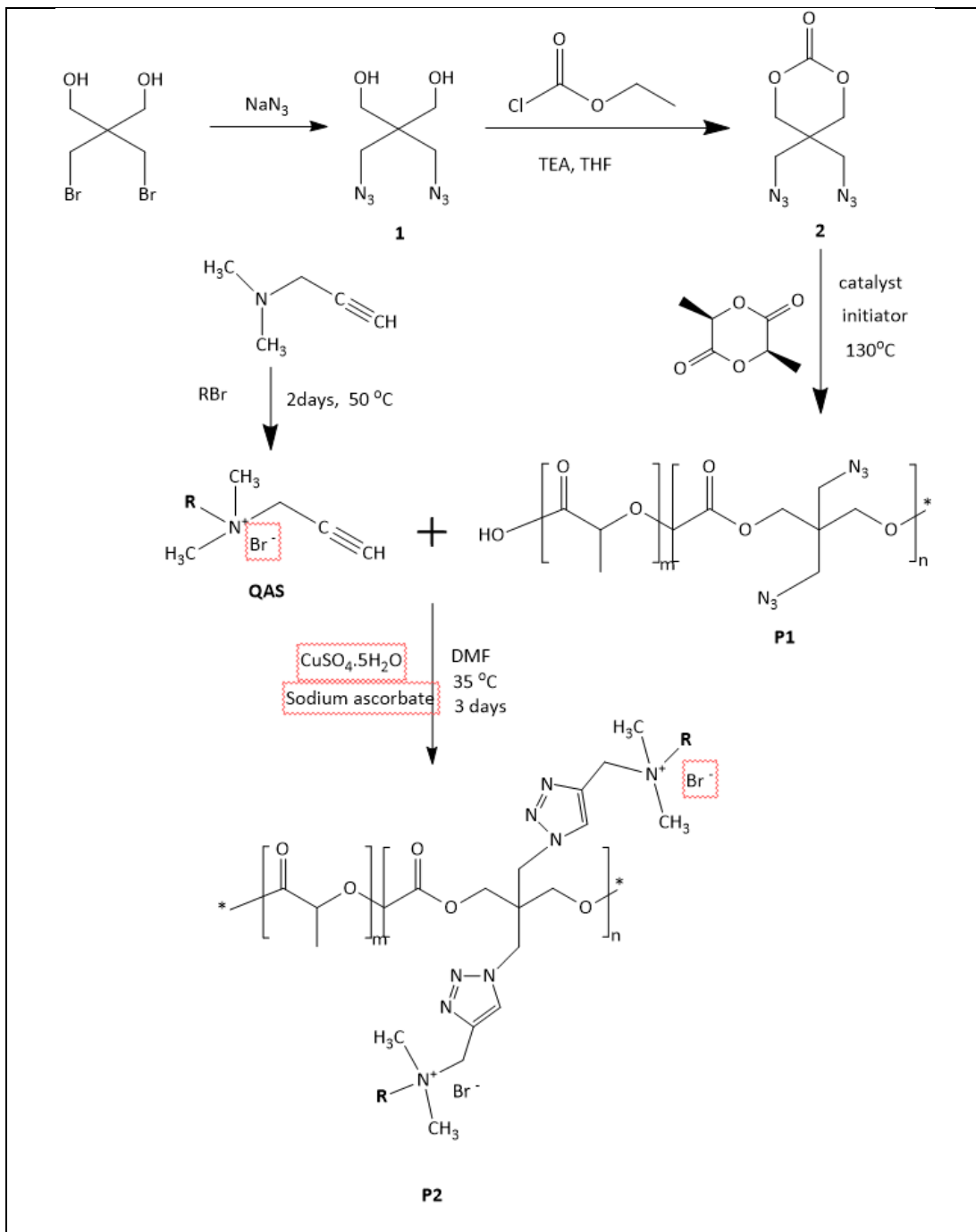


Figure 5.1. General synthesis of quaternized ammonium functionalized PLA for antimicrobial activity ($m : n \equiv 0.95 : 0.05$; $0.90 : 0.10$; $0.80 : 0.20$; $0.70 : 0.30$. $\text{R} \equiv -\text{C}_3\text{H}_7$, $-\text{C}_6\text{H}_{13}$, $-\text{C}_{12}\text{H}_{25}$).

5.1.4.Synthesis of azido carbonate monomer 2,2-bis(azidomethyl)trimethylene carbonate (ADTC) (2)

ADTC was prepared according to the literature [15], [27]. A THF solution (10 mL) of ethyl chloroformate (19.93 g, 183.65 mmol) was added to a solution of 1 (14 g, 75.27 mmol) in anhydrous THF (200 mL) over 10 min at ice-cooled temperature under argon. Dry triethylamine (21.17 g, 209.25 mmol) was added dropwise over a period of 20 min at ice-cooled temperature, too. The reaction was continued at room temperature for 5 h. The white precipitate was filtered off and the solvent was evaporated under vacuum. The crude product was purified by recrystallization from THF/ethyl ether (1:3 by volume). The recrystallization was repeated three times. White crystals (7.2 g, 45% yield) were obtained by filtration and dried under vacuum at 40 °C for 48h.

^1H NMR (CDCl_3 , 500MHz): δ 4.25 (s, $-\text{CH}_2\text{O}-$, 4H), 3.55 (s, $-\text{CH}_2\text{N}_3$, 4H). ^{13}C NMR (CDCl_3 , 125 MHz): δ 147.4, 70.5, 50.6, 36.8 (see Figure B1.2, Appendix B).

5.1.5.Preparation of *N,N*-dimethyl-*N*-prop-2-yn-1-yl dodecane-1-ammonium bromide (QAS-12C)

The synthesis of alkyne functionalized quaternary ammonium salt with aliphatic spacer 12 ($n = 12$) (QAS-12C) is given as a representative example for other aliphatic spacers ($n = 3, 6$). QAS-12C was synthesized using the method reported in the literature [13]. 1-Bromododecane (2.4 g, 9.63 mmol) was added to a round-bottom glass flask containing 15mL THF with a magnetic stir bar, followed by 0.96 g (11.55 mmol) *N,N*-dimethylprop-2-yn-1-amine (Figure 5.2). After 2 days at 50 °C, the solvent was evaporated in vacuum. The ammonium salt was dissolved in methylene chloride and purified by two precipitations in hexane. Straw yellow powder (1.8 g, 80% yield) was obtained.

^1H NMR (500 MHz, CDCl_3 , δ , ppm): δ = 4.8 (m, 2H, $-\text{CH}_2\text{N}^+$), 3.6 (m, 2H, N^+CH_2-), 3.4 (m, 6H, $-\text{CH}_3$), 2.9 (m, 1H, $-\text{C}\equiv\text{CH}$), 1.7–1.2 (m, 20H, 10 CH_2), 0.8 (m, 3H, $-\text{CH}_3$). ^{13}C NMR (CDCl_3 , δ , ppm): δ = 81 ($\text{HC}\equiv\text{C}-$), 71.5 ($\text{HC}\equiv\text{C}-\text{CH}_2\text{N}^+$), 64 (N^+CH_2), 54 ($\text{CH}_2\text{CH}_2-\text{N}^+$), 51 (CH_3N^+), 31.9, 29.6, 29.4, 29.3, 29.2, 29.1, 26.2, 22.8, 22.6 (9 CH_2), 14 (CH_3) ppm (see Figure B1.3, Appendix B).

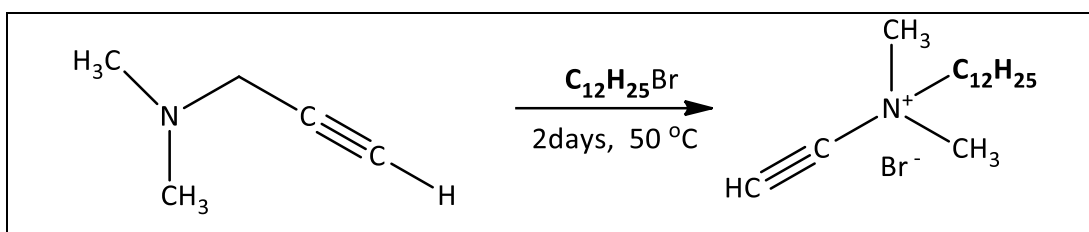


Figure 5.2: Synthesis of *N,N*-dimethyl-*N*-prop-2-yn-1-yl-dodecane-1-ammonium bromide.

5.1.6. Preparation of *N,N*-dimethyl-*N*-prop-2-yn-1-yl-hexane-1-ammonium bromide (QAS-6C)

1-Bromohexane (2.4 g, 14.54 mmol) was added to a round-bottom glass flask containing 15 mL THF with a magnetic stir bar, followed by 1.45 g (17.45 mmol) *N,N*-dimethylprop-2-yn-1-amine. After 2 days at 50 °C, the solvent was evaporated in vacuum. The ammonium salt was dissolved in methylene chloride and purified by two precipitations in hexane. Straw yellow powder (2.16 g, 90% yield) was obtained.

^1H NMR (500 MHz, CDCl_3 , δ , ppm): δ = 4.8 (m, 2H, $-\text{CH}_2\text{N}^+$), 3.6 (m, 2H, N^+CH_2-), 3.4 (m, 6H, $-\text{CH}_3$), 2.9 (m, 1H, $-\text{C}\equiv\text{CH}$), 1.7–1.2 (m, 8H, 4 CH_2), 0.8 (m, 3H, $-\text{CH}_3$).

5.1.7. Preparation of *N,N*-dimethyl-*N*-prop-2-yn-1-yl-propane-1-ammonium bromide (QAS-3C)

1-Bromopropane (3.0 g, 24.39 mmol) was added to a round-bottom glass flask containing 15 mL THF with a magnetic stir bar, followed by 2.43 g (17.45 mmol) *N,N*-dimethylprop-2-yn-1-amine. After 2 days at 50 °C, the solvent was evaporated in vacuum. The ammonium salt was dissolved in methylene chloride and purified by two precipitations in hexane. Straw yellow powder (2.70 g, 90% yield) was obtained.

^1H NMR (500 MHz, CDCl_3 , δ , ppm): δ = 4.8 (m, 2H, $-\text{CH}_2\text{N}^+$), 3.6 (m, 2H, N^+CH_2-), 3.4 (m, 6H, $-\text{CH}_3$), 2.9 (m, 1H, $-\text{C}\equiv\text{CH}$), 1.7 (m, 2H, $-\text{CH}_2$), 0.8 (m, 3H, $-\text{CH}_3$).

5.1.8. Typical Procedures for Synthesis of Copolymer poly(Lactide-co-2,2-bis(azidomethyl)trimethylene carbonate) ((LA_{0.7}-co-ADTC_{0.3}), P1)

LA and ADTC were polymerized in one pot using toluene as solvent, dodecanol as the initiator and Sn(Oct)₂ as the catalyst. The molar ratio of ADTC and LA was varied to prepare random block copolymers with different ADTC contents. For example, to prepare P(LA_{0.70}-ADTC_{0.30}), ADTC (0.847 g, 0.004 mol) and recrystallized LA (1.343 g, 0.009 mol) together with 436 μ L of dodecanol solution (0.5 mol L⁻¹ in toluene) were mixed in a Schlenk flask under argon. Finally, 66.4 μ L of Sn(Oct)₂ solution (0.1 mol L⁻¹ in toluene) was injected. The flask was evacuated and charged with argon three times. After the mixture was stirred at 130 °C for 72 h, the reaction was quenched by cooling to room temperature; the raw product was dissolved in methylene chloride and precipitated into cold hexane to obtain powder. The polymer was dried under reduced pressure at 35 °C until a constant weight was obtained (1.91 g, 87% yield).

$M_{n, GPC}$: 90800 g/mol; M_w/M_n : 1.25. FTIR (cm⁻¹): 1752 (C=O stretching), 2110 cm⁻¹ (N₃ group). ¹H NMR (500MHz, CDCl₃, δ , ppm): δ 5.17 (>CHO- in PLA); 4.24–4.05 (m, -OCH₂-C- in ADTC); 3.50–3.41 (s, N₃CH₂-C- in ADTC); 1.58 (m, >CCH₃ in PLA); 1.41 (m, 20H, -CH₂- in dodecyl), 0.82 (m, 3H, -CH₃ in dodecyl).

5.1.9. Cycloaddition of *N,N*-dimethyl-*N*-prop-2-yn-1-yl dodecane-1-ammonium bromide onto Poly(LA_{0.7}-co-ADTC_{0.3}) (P(LA_{0.7}-co-ADTC_{0.3}-QAS-12C, P2) by click chemistry

P2 was synthesized according to the literature [16], [91]. P1 (0.6 g, contains 2.19 mmol N₃) was dissolved in a minimal amount of DMF, and then synthesized alkyne-QAS-C12 (2 equivalents, 1.45 g, 4.38 mmol) was added to the solution. After the solution became fully transparent, sodium ascorbate (0.3 equivalents, 0.26 g, 0.66 mmol) was added and the reaction mixture was degassed by gently purging with oxygen-free argon for 5 min. Subsequently, the copper catalyst CuSO₄·5H₂O (0.3 equivalents, 0.33 g, 0.66 mmol) was added to the mixture and it was degassed again for 5 min. The reaction mixture was stirred at room temperature for 48 h. At the end of the reaction, the polymer was isolated by dialysis (molecular weight cut-off 500–2000) in a water solution and then *N,N,N',N'',N'''*-pentamethyldiethylenetriamine was

added dropwise to this solution to remove the residual copper ions by complex formation. Finally, the obtained polymer was dried under vacuum at 40 °C to a constant weight (1.86 g, 90% yield).

FTIR (cm^{-1}): 1752 (C=O stretching), 1608 (N=N stretching). ^1H NMR (DMSO): δ 8.60–8.45 (s, ^1H , CH of triazole ring, g), 5.23 (>CHO– in PLA, a), 1.47 (m, >CCH₃ in PLA, b), 4.64 (m, –OCH₂–C– in ADTC, c), 4.21 (s, N₃CH₂–C– in ADTC, d), 5.04 (m, 2H, CH₂N⁺, h), 3.02 (m, 6H, 2CH₃, i), 3.19 (m, 2H, N⁺CH₂, j), 0.82 (m, 3H, CH₃, f, m).

5.1.10. Antimicrobial assessment

The antimicrobial activity test was performed in a 96-well plate. Plates were coated by dispensing 100 μL of this polymeric solution (10 mg mL^{-1} in 100% methanol) to the wells. The plates were then transferred into a vacuum oven and the solvent was evaporated at 35 °C. Each experiment was performed in triplicate and wells coated only with methanol were also added to the experiments. Gram-negative (*Escherichia coli* ATCC 25922) and Gram-positive (*Staphylococcus aureus* ATCC 29213) bacteria were used for the antimicrobial activity tests. Bacteria were grown overnight on nutrient agar plates. A loopful of bacteria were suspended in saline solution (0.9% NaCl). The turbidity of the bacterial suspensions was adjusted to 0.25 and 0.40 optical density at 600nm (OD₆₀₀) for *S. aureus* and *E. coli* respectively (approximately equivalent to 1.5×10^8 cfu mL^{-1}). One hundred microlitres of freshly prepared bacterial suspensions were transferred to wells coated with polymeric solution. The plates were incubated at room temperature for 16 h. After incubation, the viable cell count of bacterial suspensions was determined by the drop plate method [83].

5.2. Preparation of Antimicrobial PLA Film

5.2.1. Synthesis of *N,N*-dimethyl-*N*-prop-2-yn-1-yl dodecane-1-ammonium bromide onto Poly(LA_{0.7}-co-ADTC_{0.3}) (P(LA_{0.7}-co-ADTC_{0.3}-QAS-12C, P2)

Antimicrobial polymer, P2, was employed for spray coating method to impart antimicrobial property to the neat PLA film. The synthesis of P2 was explained in part 5.1.9.

5.2.2. Film Preparation

PLA films were prepared by the solvent-casting technique. PEG 1000 was used as a plasticizer. 3.43 g PLA pellets were added into 26 ml chloroform in a 100 ml glass beaker, followed by the addition of 0.38 g PEG1000. The final concentration of PEG in PLA was 10% w/w. The solution was stirred at room temperature until all PLA pellets were dissolved. Subsequently, the film solutions were cast into petri dishes (diameter, D=14 cm) and the solvent slowly evaporated. Then, films were peeled from the glass plate.

5.2.3. Coating One Surface of Films with Antimicrobial Polymer

Spray coating method [148] was used for producing thin antimicrobial coatings on one side of PLA films. First of all, only acetone was added to the spray bottle and it was calculated how much acetone was sprayed after once spray. For this calculation, acetone was sprayed 10 times and then the loss in mass was calculated. This process was repeated 3 times. Finally, it was calculated that 0.75 g of acetone was sprayed onto the film surface per once-spray. Subsequently, 5 g antimicrobial polymer (P1) was dissolved in 100 g of acetone. So,

$$0.75 * 0.05 = 0.0375 \text{ g} \quad (5.1)$$

polymer was sprayed onto the film per once-spray. The amount actually intended to be on the film surface is 0.001 g cm⁻². Film area is calculated using Equation (5.2).

$$A = \pi r^2 \quad (5.2)$$

$$A = 3.14 \left(1\frac{1}{2}\right)^2 = 154 \text{ cm}^2 \quad (5.3)$$

Therefore, the amount of antimicrobial polymer intended to be on the entire surface of the film is,

$$0.001 \text{ g cm}^{-2} * 154 \text{ cm}^2 = 0.154 \text{ g} \quad (5.4)$$

From this result, It can be calculated how many times the entire surface of the film should be sprayed to obtain the desired amount of antimicrobial polymer on the film surface,

$$\frac{0.154}{0.0375} \approx 5 \text{ times spray} \quad (5.5)$$

After having this result, three films were prepared and their surfaces were sprayed 5, 15 and 25 times, respectively. These films were encoded as PLA+5, PLA+15 and PLA+25. Besides, reference film encoded as PLA (Ref) which not coated with antimicrobial polymer was prepared for comparison. Lastly, antimicrobial activity and the thermal, mechanical and barrier properties of all these films were investigated.

5.2.4. Fourier Transform Infrared (FTIR) Analysis

Fourier transform infrared (FTIR) spectra were measured via the attenuated total reflectance (ATR) method on a Perkin Elmer Paragon 1000 spectrometer equipped with PIKE MIRacle™ diamond ATR attachment at wavenumbers ranging from 650 to 4000 cm^{-1} . All the readings were performed at ambient temperature.

5.2.5. Antimicrobial Assessment

Antimicrobial activity of the films (PLA (Ref), PLA+5, PLA+15 and PLA+25) was investigated via contact-active method. Films were equally cut to pieces (1 cm^2) and freshly prepared bacterial (*Staphylococcus aureus*) solution in saline (total number of

bacteria was approx. 10^6 cfu) was dropped on the surface. Film pieces left to dry for 3 hours under sterile conditions. Films then transferred to Eppendorf tubes containing 0.5 ml LB medium. The tubes were vortexed for 30 seconds and then subjected to sonication (using Bioruptor® Pico sonication device, Diagenode Inc./USA) for 2 minutes followed by additional vortexing for 30 seconds. Bacteria in the resulting sonicate fluid were enumerated by routine microbiologic techniques [84].

5.2.6. Mechanical Properties

Tensile tests were carried out with an Instron universal testing machine (Model 3345) (Figure 5.3) according to the ASTM standard method D882–88. Specimen samples were cut into 8 cm x 2 cm strips from film samples. The crosshead speed was 10 mm/min. Five specimens of each sample were tested and the mean value was reported.

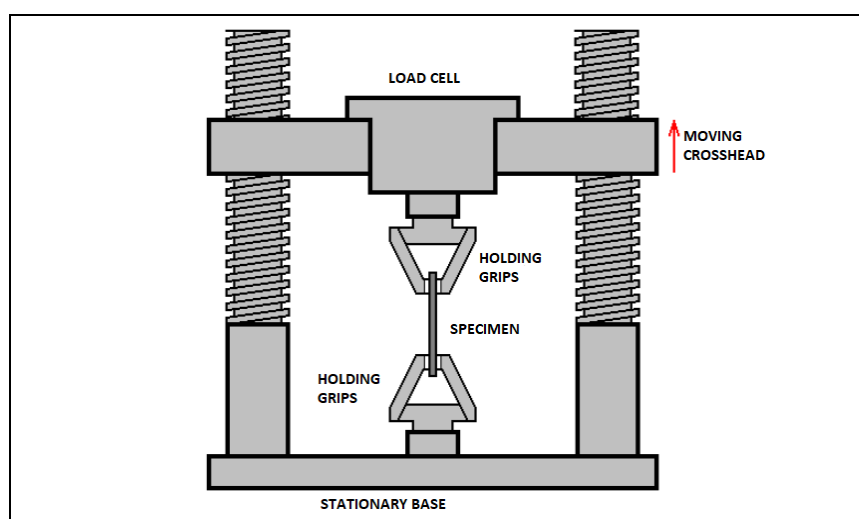


Figure 5.3: Tensile Test Machine.

Stress-strain curves were obtained after tensile testing (Figure 5.4). The tensile strength (TS), elongation at break (%E), and Young's modulus (E) of the films were measured. Tensile strength (TS) was calculated by dividing the maximum load on the film before failure by the cross-sectional area of the initial specimen. Young's (Elastic) modulus (E) was determined according to the slope of the stress /strain curve in the

linear range (Figure 5.4). Percentage elongation (E) was defined as the percentage change in the length of the specimen to the original length between the grips [85], [86].

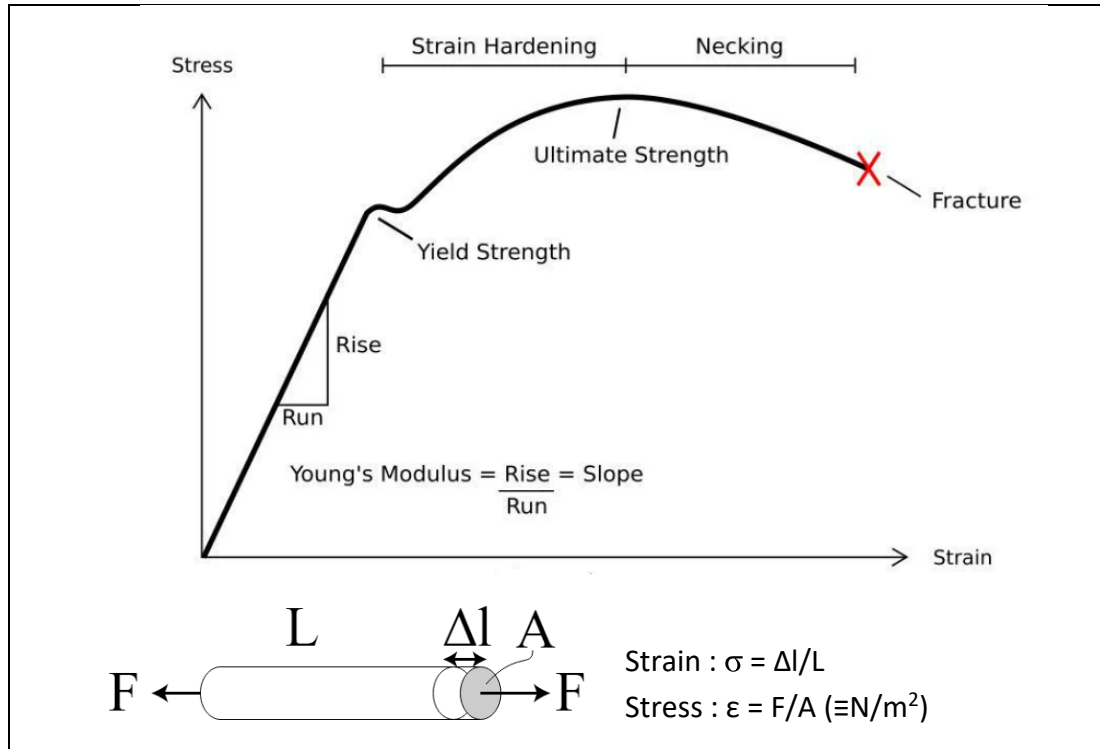


Figure 5.4: The stress-strain curve obtained after the tensile test.

5.2.7. Differential Scanning Calorimetry (DSC)

The differential scanning calorimetry (DSC) of samples was performed using DSC 8500 (Perkin Elmer) instrument under nitrogen atmosphere. The samples were first heated from 25 to 150 °C at a scan rate of 10 °C min⁻¹, then cooled to 0 °C and finally heated again to 300 °C at a scan rate of 10 °C min⁻¹. Glass transition temperature (T_g), melting temperature (T_m) and enthalpies of melting (ΔH_m) and cold crystallization (ΔH_c) of PLA (Ref), PLA+5, PLA+15 and PLA+25 were determined via DSC thermograms. The percent crystallinity ($X_c\%$) of the samples was calculated according to Equation (5.6) [85]:

$$X_c \% = \frac{(\Delta H_m - \Delta H_c)}{\Delta H_m^c} \times 100 \quad (5.6)$$

where ΔH_m is the enthalpy of fusion, ΔH_c is the enthalpy of crystallization, and ΔH_m^c is the enthalpy of fusion of purely crystalline PLA (106 J/g).

5.2.8. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis tests were performed in TGA/SDTA 851 (Mettler Toledo) thermogravimetric analyzer. Approximately 5-mg specimens were weighed in aluminum pans and heated from room temperature up to 700 °C at a heating rate of 10 °C min⁻¹ under nitrogen atmosphere. Weight losses of samples were measured as a function of temperature.

5.2.9. Water Vapor Permeability (WVP)

The WVP of films was measured gravimetrically according to the ASTM E96 for *Desiccant Method* with some modifications [85], [87], [88]. This test was taken place only for PLA (Ref) and PLA+25 films. Circular film samples were mounted and sealed on the open mouth of cylindrical cups containing 40 g of anhydrous calcium chloride (0% relative humidity, RH), leaving an air gap of 1 cm between the film underside and the desiccant. Then, each cup was placed in desiccators containing a saturated potassium nitrate solution (93% RH). The cups were weighed every day and nine weight measurements were taken over 9 days. Changes in the weight of the cups were recorded to the nearest 0.0001 g and plotted as a function of time. The slope of each line was calculated by linear regression ($r^2 > 0.99$), and the water vapor transmission rate (WVTR) was calculated from the slope of the straight line (g/s) divided by the cell area (m²). The exposed area was 0.0028 m². After the permeation tests, the film thickness was measured and the WVP (g·m/m²·s·Pa) was calculated as follows (Equation 5.7) [87]:

$$WVP = \left[WVTR / S (R_1 - R_2) \times D \right] \quad (5.6)$$

where S is the saturation vapor pressure of water (Pa) at the test temperature (25°C), R_1 is the RH in the desiccator, R_2 is the RH in the permeation cell and D is the film thickness (m). For each measurement, three replications were made.

5.2.10. Oxygen Permeability

Oxygen permeability (OP) of PLA (Ref) and PLA+25 films was measured by an Ox-Tran 2/21 ML modular system (Modern Controls Inc., Minneapolis, MN, USA) according to standard method ASTM D3985. Each film sample was placed on an aluminum mask with an open testing area of 5 cm² and placed in the test cell. During the test at 23 °C and 0± 1% RH, one side of film sample was exposed to 98% N₂ ± 2%H₂ flow and the other side was exposed to high purity dry air flow. Oxygen permeability was calculated by dividing O₂ transmission rate by the difference in O₂ partial pressure between both sides of the film (101 kPa) and multiplying by the average thickness of the film (μm) [89], [90]. Measurements were performed in duplicate and the units for OP were cm³.mm/m².day.atm.

5.2.11. Scanning Electron Microscopy (SEM)

The morphological monitoring of the obtained film surfaces was characterized by using SEM (Philips XL30 FEG, Oregon, USA). Also, phase morphologies of film were investigated by means of SEM (JEOL JSM-6060). The samples for this analysis were cryogenically fractured in liquid nitrogen and sputter coated with gold before observations to prevent arching.

6. RESULTS AND DISCUSSION

6.1. Synthesis of Quaternized Ammonium Functionalized PLA

6.1.1.Characterization of Azido Carbonate Monomer 2,2-bis(azidomethyl)trimethylene carbonate (ADTC) (2)

The cyclic carbonate monomer containing azido groups, ADTC (**2**), was synthesized from 2,2-bis(bromomethyl)propane-1,3-diol (**1**) in two steps according to procedures given in the literature [27], [28]. The structures of (**1**) and (**2**) was confirmed by ^1H -NMR and FT-IR spectral analysis.

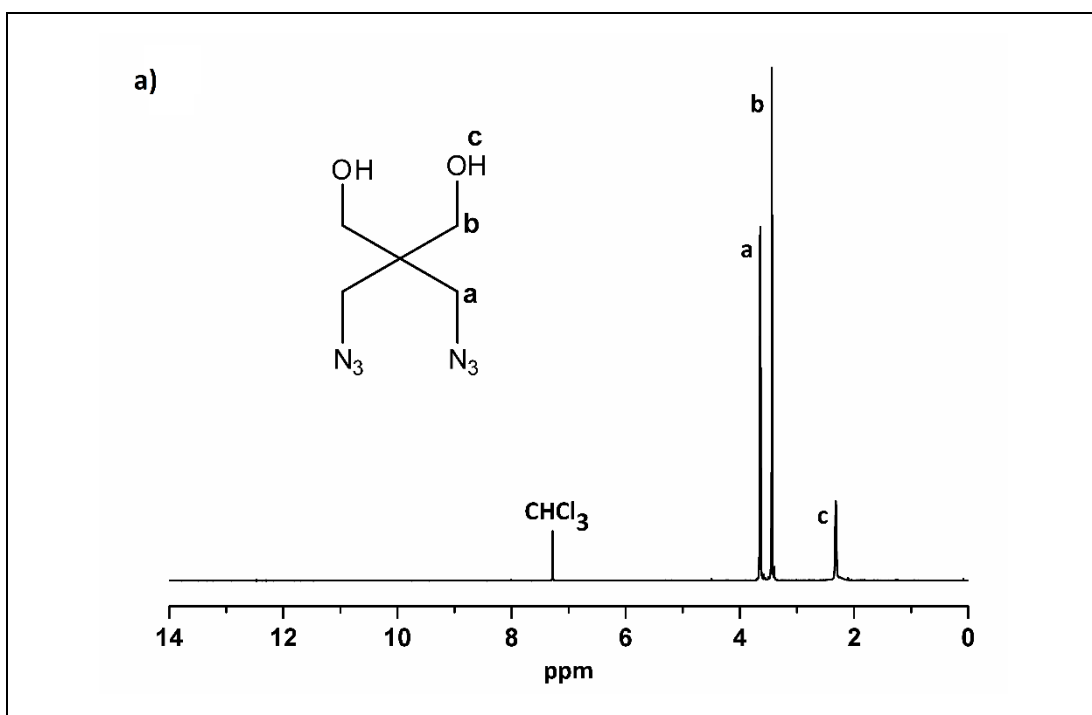


Figure 6.1: a) ^1H NMR spectra, and b) FTIR spectrum of 2,2-bis(azidomethyl)propane-1,3-diol (**1**).

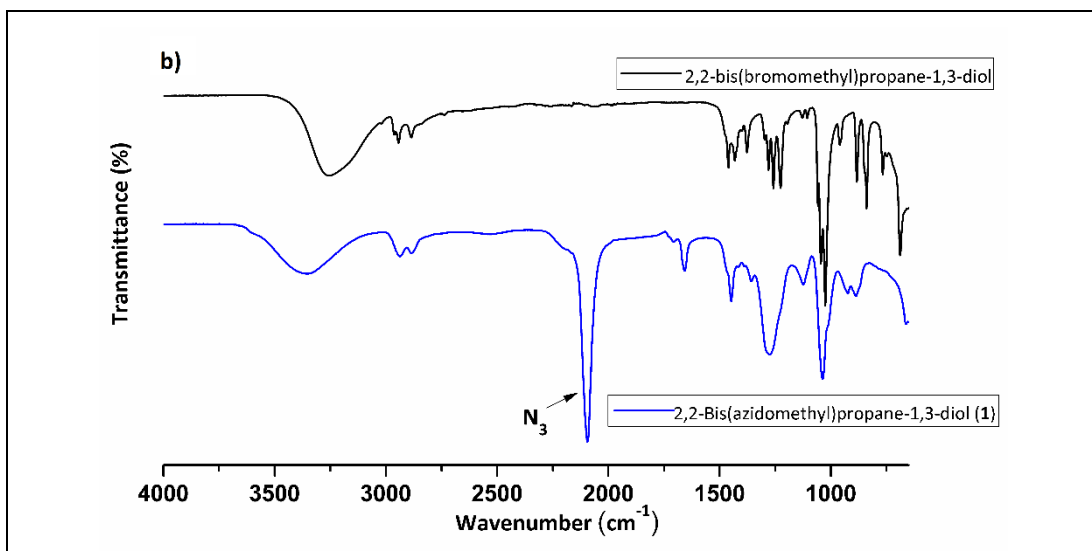


Figure 6.1: Figure of Continuation.

In the ^1H NMR spectra of **1** (Figure 6.1 (A)), protons ($-\text{CH}_2\text{N}_3$, 4H, H_a), ($-\text{CH}_2\text{OH}$, 4H, H_b) and ($-\text{OH}$, 2H, H_c) gave signals at 3.62, 3.41 and 2.35 ppm, respectively. Also, in the FT-IR spectrum of **1** (Figure 6.1 (B)), the signal observed at 2095 cm^{-1} clearly indicates the presence of azide functional groups in the chemical structure of **1**.

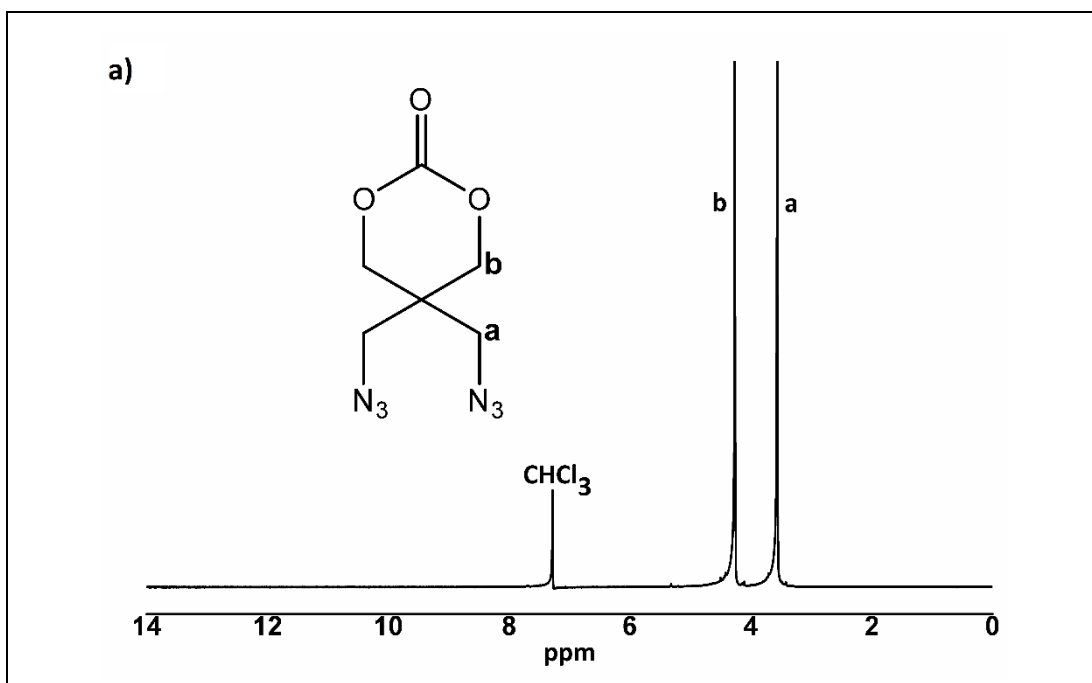


Figure 6.2: a) ^1H NMR spectra, and b) FTIR spectrum of 2,2-bis(azidomethyl)trimethylenecarbonate (ADTC).

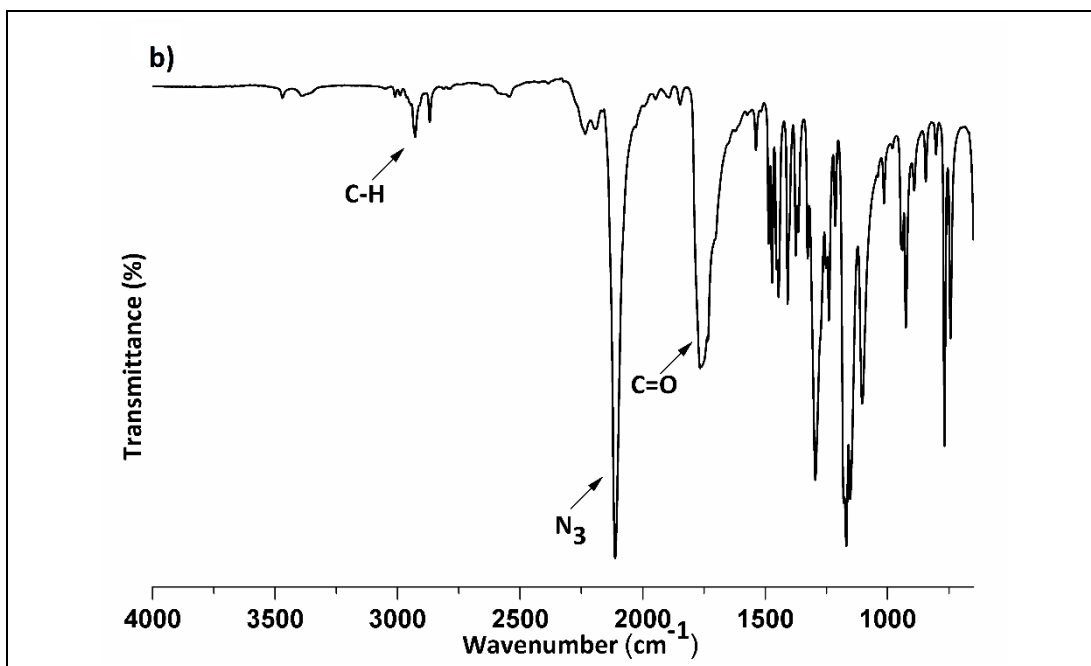


Figure 6.2: Figure of Continuation.

In the ^1H NMR spectra of **2** (Figure 6.2 (A)), protons ($-\text{CH}_2\text{N}_3$, 4H, H_a) , ($-\text{CH}_2\text{OH}$, 4H, H_b) and gave signals at 3.55 and 4.25 ppm, respectively. The strong peak at 1750 cm^{-1} in the FTIR spectra corresponds to the carbonyl groups and strong peak at 2110 cm^{-1} corresponds to the azido (N_3) group [Figure 6.2 (B)], also indicating successful synthesis of the monomer.

6.1.2. Characterization of Alkyne Functional Quaternary Ammonium Salts

Alkyne functionalized quaternary ammonium salts having various alkyl chain lengths (QAS-3C, QAS-6C, QAS-12C) were synthesized and the structures were confirmed by ^1H NMR. It was shown that the signal of the methylene protons that observed at 1.7–1.2 ppm (H_f) increased while the length of the alkyl group on the ammonium salts increased.

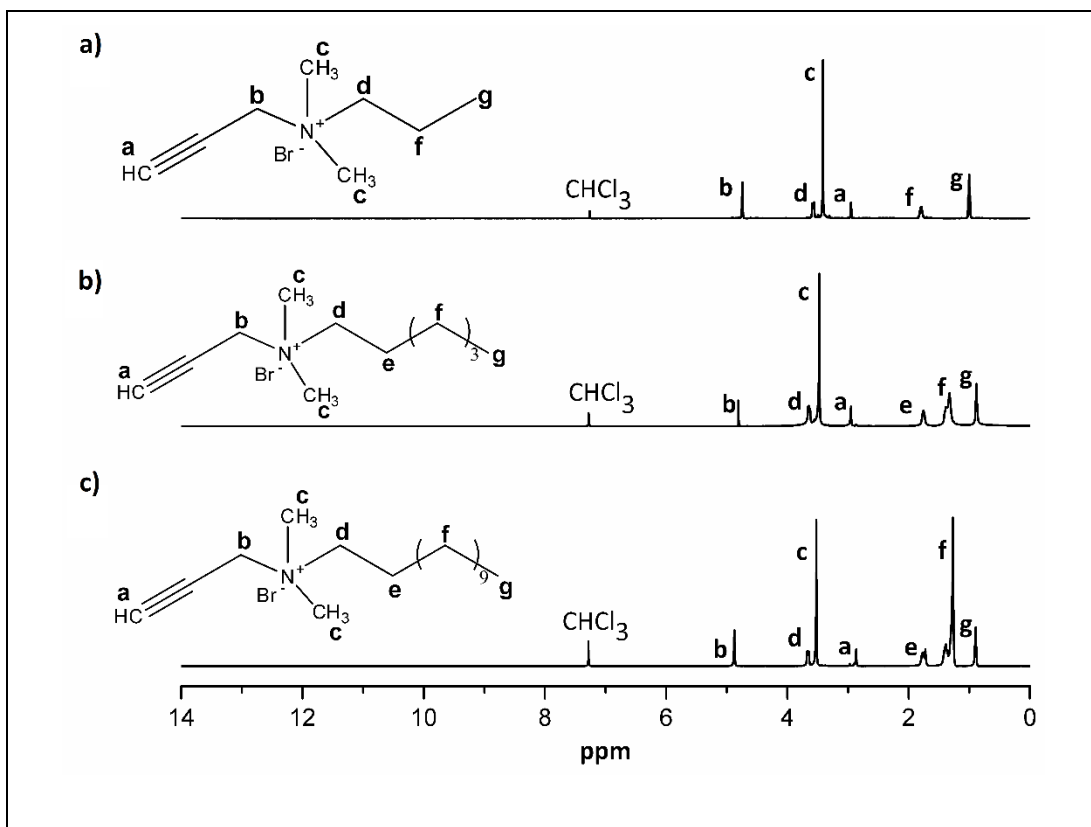


Figure 6.3: ^1H NMR spectra of a) QAS-3C; b) QAS-6C; c) QAS-12C.

6.1.3.Characterization of Poly(LA_{0.7-co}-ADTC_{0.3}), P1 and P(LA_{0.7-co}-ADTC_{0.3})-QAS-12C, P2

The poly(lactide-*co*-2,2-bis(azidomethyl)trimethylene carbonate) (P(LA-*co*-ADTC)) copolymers were prepared by the ROP of LA and ADTC in the presence of 1-dodecanol as protic co-initiator and $\text{Sn}(\text{Oct})_2$ as catalyst. Figure 6.4 gives the ^1H NMR spectra of copolymer P(LA-*co*-ADTC) with 30 mol% of ADTC monomer (**P1**). The CH and CH_3 protons of LA repeat units show chemical shifts at 5.1 and 1.56 ppm according to PLA. The signal H_c from 4.2 to 4.3 ppm is assigned to $-\text{CO}-\text{O}-\text{CH}_2-$ protons of repeat units of ADTC. Peaks H_d at 3.5 ppm are assigned to $\text{N}_3-\text{CH}_2-\text{C}-$ protons of repeat units of ADTC [30].

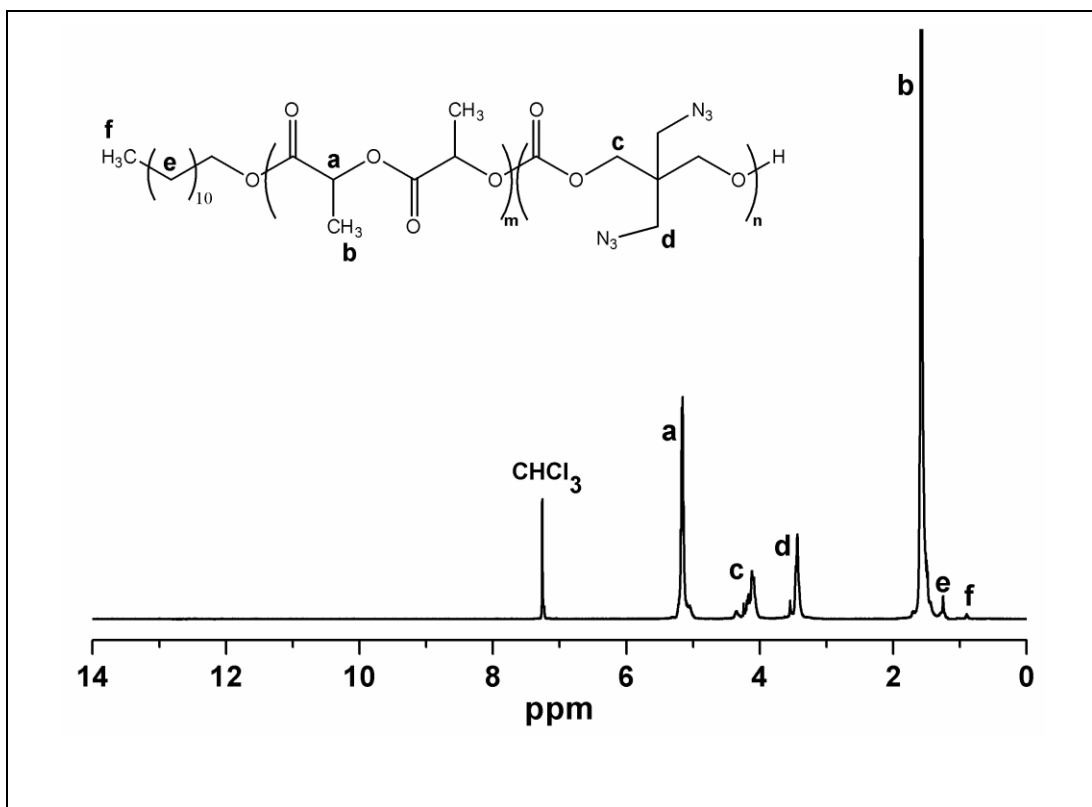


Figure 6.4: ^1H NMR spectrum of $\text{P}(\text{LA}_{0.7}\text{-co-ADTC}_{0.3})$ (the subscript numbers 0.7 and 0.3 refer to the molar ratio of LA and ADTC in the feed).

Alkyne functionalized quaternary ammonium salts having various alkyl chain lengths were synthesized and the structures were confirmed by ^1H NMR (see Figure 6.3). Then, they were introduced into the $\text{P}(\text{LA-co-ADTC})$ backbone by the click chemistry technique in mild conditions with the CuSO_4 -sodium ascorbate catalytic system in DMF. The progress of the reaction was monitored via FTIR spectral analysis (Figure 6.5). Observation of the disappearance of the azide signal at 2104 cm^{-1} and occurrence of a new absorption peak at 1608 cm^{-1} , characteristic of the vibration of the unsaturated triazoles, confirmed a successful click reaction [91].

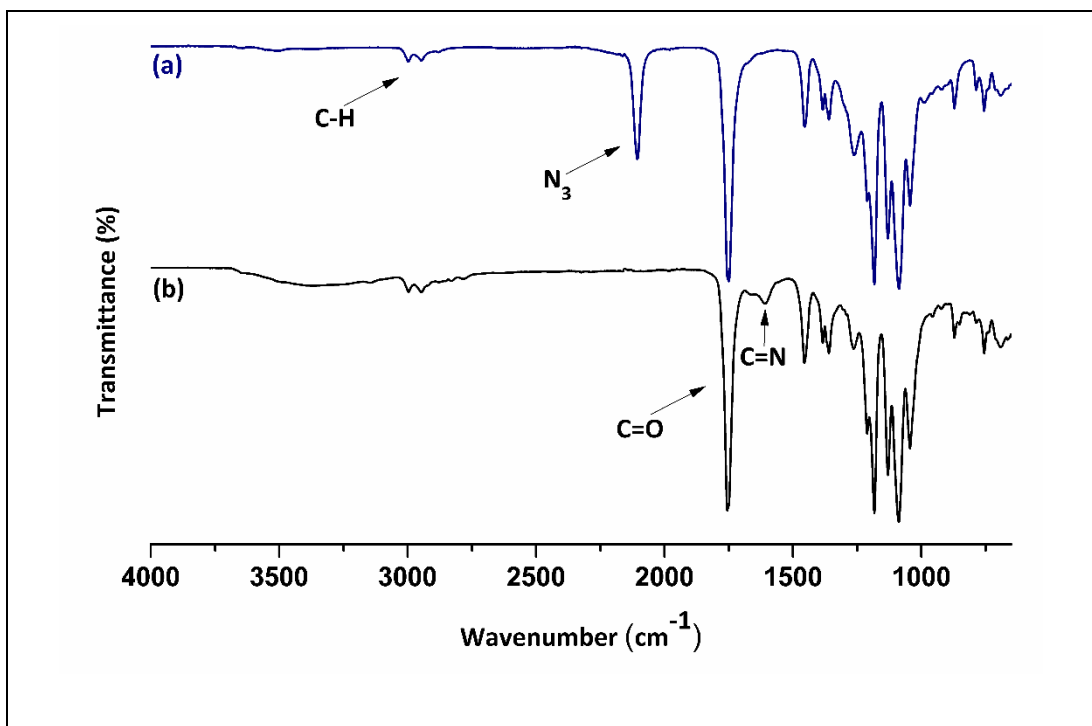


Figure 6.5: FTIR spectra of a) P1 (P(LA_{0.7}-co-ADTC_{0.3})) and b) P2 (P(LA_{0.7}-co-ADTC_{0.3}-QAS-12C)).

After the copolymers were dried under vacuum, the ^1H NMR spectra were measured in DMSO- d_6 . The characteristic signal of the proton of the triazole ring at 8.5 ppm (H_e) demonstrates a successful cycloaddition reaction. The assignment of the other resonances is shown in Figure 6.6. Also, it was observed that the intensity of the NMR resonance signals at 1.26 ppm (H_j) increased proportionally to the increase in the length of the substituted alkyl chain. As beforementioned, this conclusion can be supported by ^1H NMR spectra of the synthesized quaternary ammonium salts by comparing proton peaks H_f (see Figure 6.3).

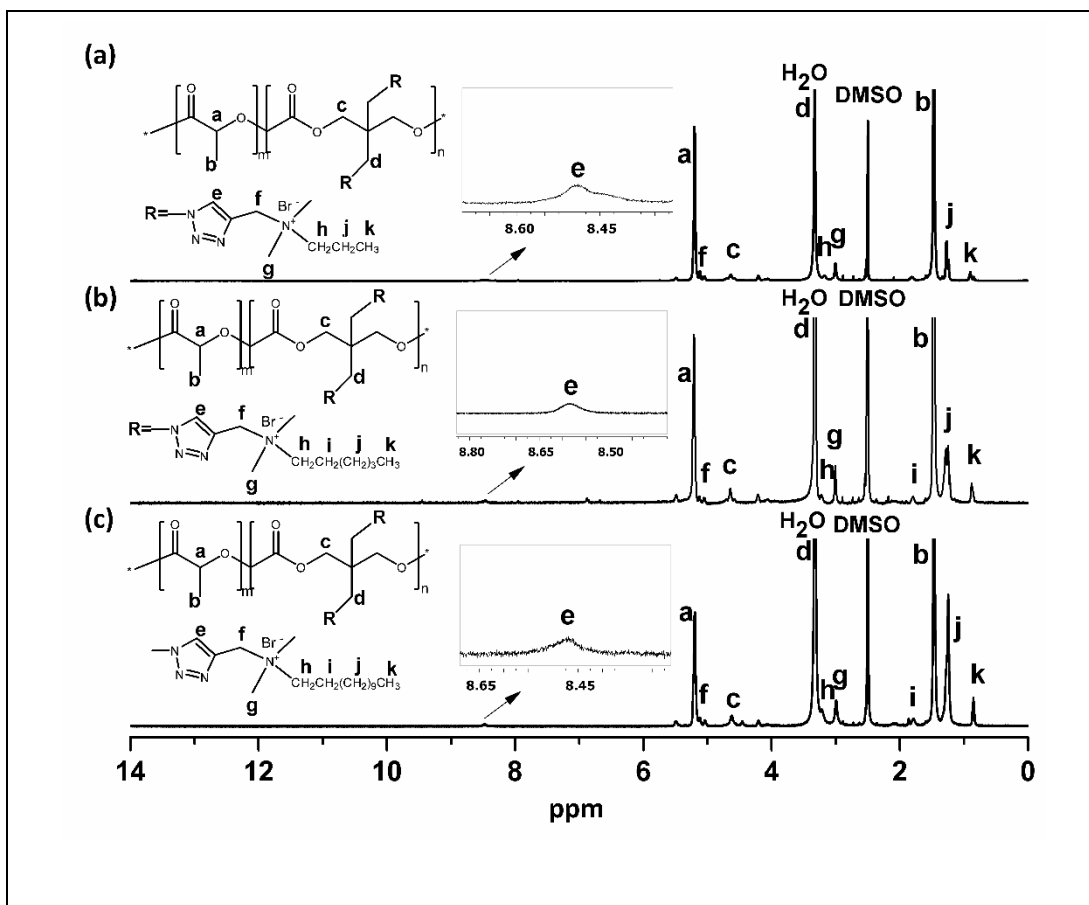


Figure 6.6: ^1H NMR spectra of a) $\text{P(LA}_{0.7}\text{-co-ADTC}_{0.3}\text{-QAS-3C)}$, b) $\text{P(LA}_{0.7}\text{-co-ADTC}_{0.3}\text{-QAS-6C)}$ and c) $\text{P(LA}_{0.7}\text{-co-ADTC}_{0.3}\text{-QAS-12C)}$ (QAS-3C, QAS-6C and QAS-12C refer to *N,N*-dimethyl-*N*-prop-2-yn-1-yl(propane, -hexane, -dodecane)-1-ammonium bromide, respectively).

The composition, molecular weights and thermal data of the copolymers are summarized in Table 6.1. The composition of the copolymers can be calculated from the integral ratio of H_b and H_c for the signal at 1.56 ppm and the 4.2–4.3 ppm peaks, respectively [28]. The data in Table 6.1 indicate that the common catalyst, Sn(Oct)_2 , is efficient for the ROP of LA with ADTC and the copolymer composition can be well controlled by the feed ratio [92]. The theoretical molecular weight ($M_{n,\text{th}}$) of P(LA-co-ADTC) was calculated from the feed ratio and yield. The number-average molecular weights (M_n) of the copolymers were estimated by GPC and the related data are summarized in Table 6.1. The copolymers had low polydispersity values (M_w/M_n), indicating that the purified polymerization products contained only the desired polymers. The number-average molecular weights ($M_{n,\text{NMR}}$) of the copolymers could also be calculated by comparing the integral ratio of methine protons of PLA with the

resonance peaks at $\delta = 1.57$ ppm, the methylene protons of ADTC with the resonance peaks at $\delta = 4.2\text{--}4.3$ ppm and the methylene protons of the initiator with the resonance peaks at $\delta = 1.26$ ppm, from their ^1H NMR spectra (Table 6.1). As shown in Table 6.1, the molecular weight measured by GPC ($M_{n,\text{GPC}}$) is close to $M_{n,\text{th}}$ and $M_{n,\text{NMR}}$ indicating good control of the ROP.

Table 6.1: Information about copolymerization of LA and ADTC Catalyzed by $\text{Sn}(\text{Oct})_2^{\text{a}}$.

Product	ADTC In product (mol%)	$M_{n,\text{th}}$ (g/mol) ^b	$M_{n,\text{NMR}}$ (g/mol) ^c	$M_{n,\text{GPC}}$ (g/mol) ^e	M_w/M_n	T_g (°C) ^f	$T_{d,\text{onset}}$ (°C) ^g	$T_{d,\text{max}}$ (°C) ^h	Char Yield (%) ⁱ
P(LA _{0.95} -co-ADTC _{0.05})	4	10300	11000	9700	1.21	41.95	214.25	297.39	3.08
P(LA _{0.90} -co-ADTC _{0.10})	8	10100	10400	9100	1.28	37.43	219.38	280.57	3.23
P(LA _{0.80} -co-ADTC _{0.20})	18	10000	10200	8800	1.33	36.81	223.05	269.96	7.62
P(LA _{0.70} -co-ADTC _{0.30})	25	9800	10100	7900	1.42	29.51	218.33	253.14	11.15
P(LA _{0.70} -co-ADTC _{0.30} - QAS-12C)	25	n.d. ^d	n.d. ^d	n.d. ^d	n.d. ^d	51.94	180.12	265.57	18.54

^a Polymerization conditions: bulk, 130 °C, 72 h. The molar ratio of $\text{Sn}(\text{Oct})_2$ to monomer (LA + ADTC) was 1/2000.

^b $M_{n,\text{th}} = \frac{[\text{ADTC}]_0 \cdot 212.17 + [\text{LA}]_0 \cdot 144.13}{[I]_0} \cdot \text{Yield}$, where $[\text{ADTC}]_0$, $[\text{LA}]_0$, and $[I]_0$ are the initial molar concentrations of ADTC, LA, and initiator in the reaction mixture.

^c $M_{n,\text{NMR}} [\text{P(LA-co-ADTC)}] = \text{MW of the initiator} + \frac{1/6 \cdot I_b \cdot 144.13 + 1/4 \cdot I_c \cdot 212.17}{1/20 \cdot I_e}$, where I is the intensity of corresponding peak in NMR spectrum.

^d Not determined.

^e $M_{n,\text{GPC}}$, and M_w/M_n were obtained from GPC experiments based on linear polystyrene standards using tetrahydrofuran (THF) as the eluent.

^f Determined by DSC with a heating rate of 10 °C min⁻¹ under nitrogen flow.

^g T_{onset} denotes the onset decomposition temperatures of the polymers.

^h T_{max} designates the temperature that corresponds to maximum rate of weight loss.

ⁱ The percent of weight remained at 700°C.

The thermal phase transition temperatures (T_m and T_g) and thermal stability of the polymers were investigated via DSC and TGA experiments, respectively. The thermal stability of all the synthesized polymers was investigated by TGA under a nitrogen atmosphere at a heating rate of 10 °C min⁻¹ from room temperature to 700

°C (Figure 6.7 and Figure 6.8). The data related to their onset ($T_{d,onset}$) and maximum thermal decomposition temperatures ($T_{d,max}$) as well as their percentage char yields are summarized in Table 6.1. According to the TGA results, whereas the onset decomposition temperatures of the copolymers did not change significantly, it was observed that $T_{d,onset}$ decreased on adding a quaternary ammonium group with dodecyl chain length onto PLA containing 30 mol% ADTC (Figure 6.8). On the other hand, the maximum thermal decomposition temperatures of the copolymers decreased slightly with increasing ADTC content, but $T_{d,max}$ increased after adding the quaternary ammonium group to the polymer (Table 6.1 and Figure 6.7). Also, the percentage char yields of the copolymers were increased, even when the quaternary ammonium group was substituted on P(LA_{0.7-co}-ADTC_{0.3}) (Table 6.1).

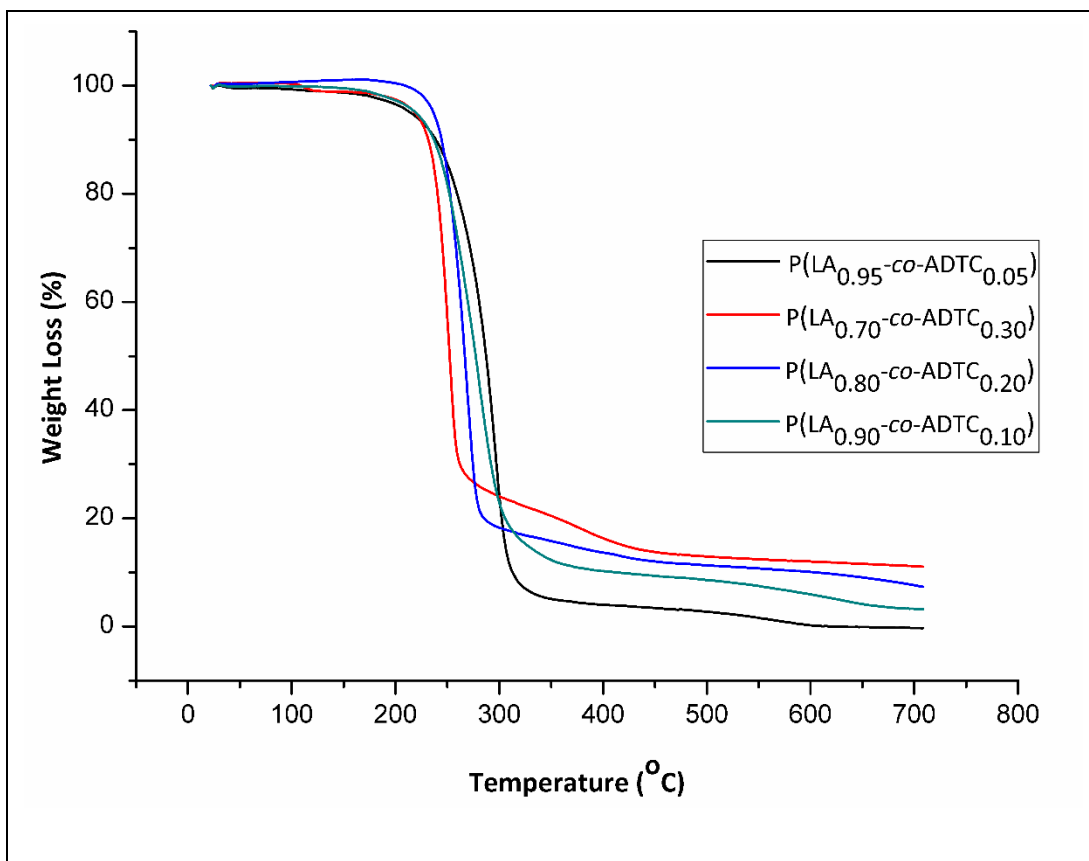


Figure 6.7: TGA thermograms of the azido-functionalized PLA copolymers.

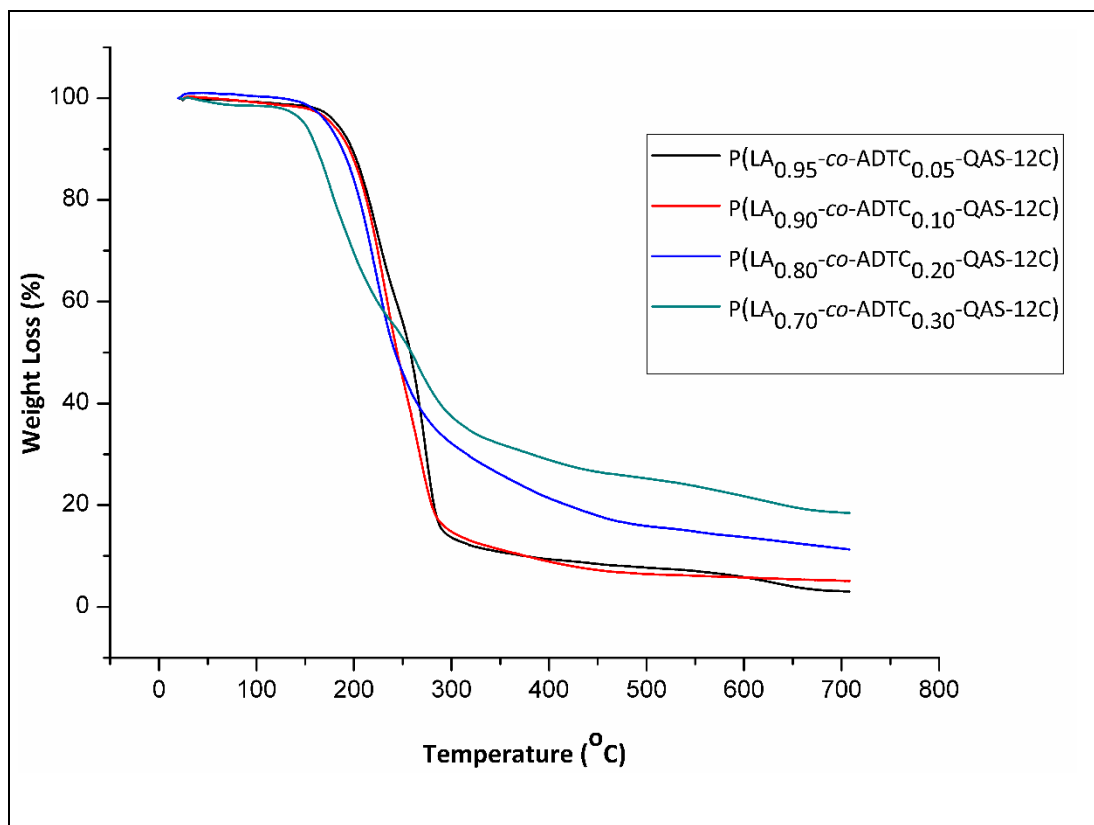


Figure 6.8: TGA thermograms of the PLA copolymers containing quaternary ammonium salt substituted by dodecyl groups.

The DSC technique was used to determine the thermal transitions, such as T_g , of all the synthesized copolymers. Figure 6.9 shows the DSC curves of the PLA copolymers containing ADTC with different molar ratios and containing 30 mol% of quaternary ammonium group with dodecyl chain length. Also, the related data are given in Table 6.1. The copolymers changed from crystalline to amorphous as the percentage of ADTC content increased (Figure 6.9). Besides, the glass transition temperature of the copolymer containing 30 mol% ADTC was increased after attachment of the quaternary ammonium group on PLA. This situation was supported by Figure 6.10. It is clearly seen that attachment of ammonium side groups considerably increased T_g of the PLA copolymers.

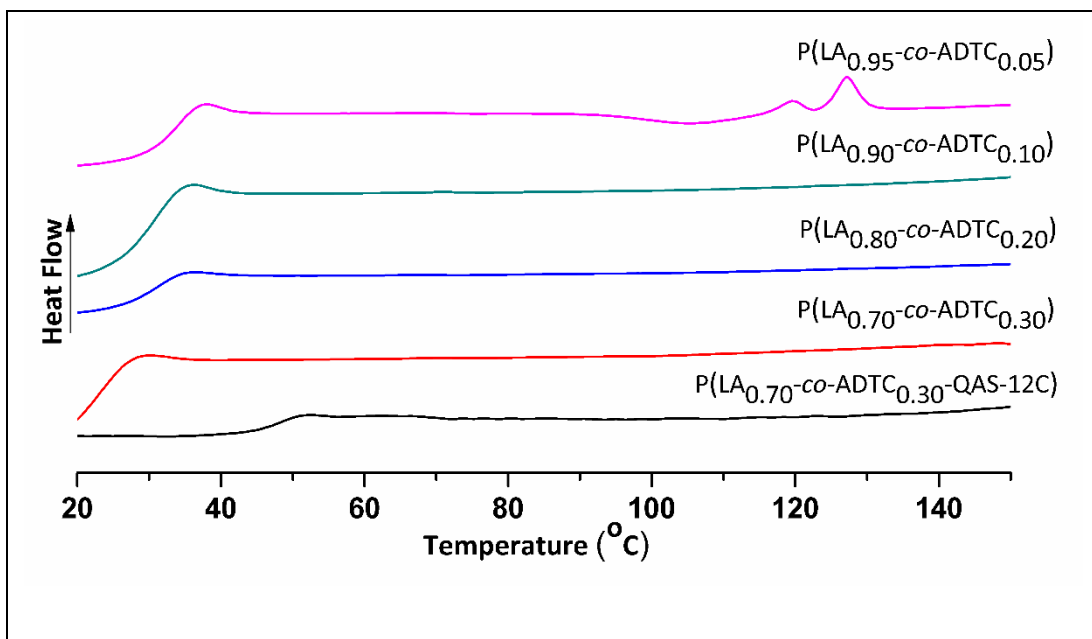


Figure 6.9: DSC curves of the azide functional PLA copolymers in the second heating runs.

The molecular weights of polymers substituted with quaternary ammonium groups could not be estimated by GPC because they did not dissolve in THF, and this result further confirmed that click post-modification on PLA was successfully carried out with substantially no degradation in molecular weight.

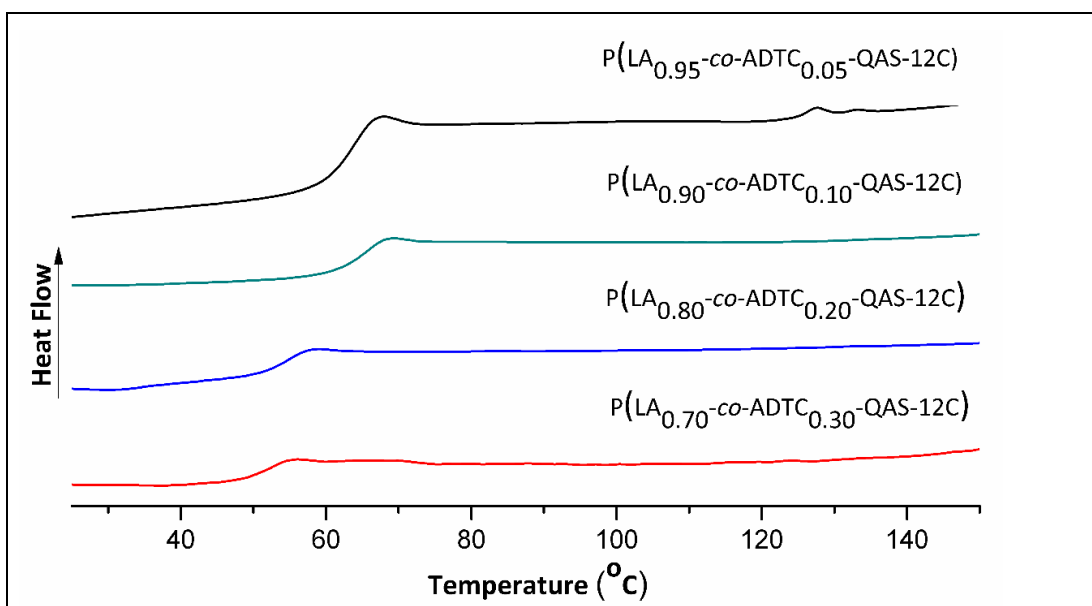


Figure 6.10: DSC curves of the PLA copolymers containing quaternary ammonium salt substituted by dodecyl groups in the second heating runs.

In addition, we investigated the polymer surface obtained by using SEM-EDX to evaluate elemental mapping analysis. The recorded EDX mapping images of the P(LA_{0.95}-ADTC_{0.05}-QAS-12C) surface are shown in Figure 6.11. From the figure, the EDX mapping spectra of P(LA_{0.95}-ADTC_{0.05}-QAS-12C) consist of carbon (C), oxygen (O), nitrogen (N) and bromine (Br) elements. The EDX mapping results show the presence of bromine in the polymer after the click reaction. As shown in the EDX mapping of P(LA_{0.95}-ADTC_{0.05}-QAS-12C) in Figure 6.11, the presence of the bromine element clearly confirms the completeness of the click reaction. Also, according to this mapping analysis, copper (Cu) element was not found in the obtained polymer. This result is evidence of the absence of residual Cu ions in the polymer samples after the copolymers were dialysed. The presence of Cu in the polymer samples is not desirable due to the bactericidal properties of Cu ion.

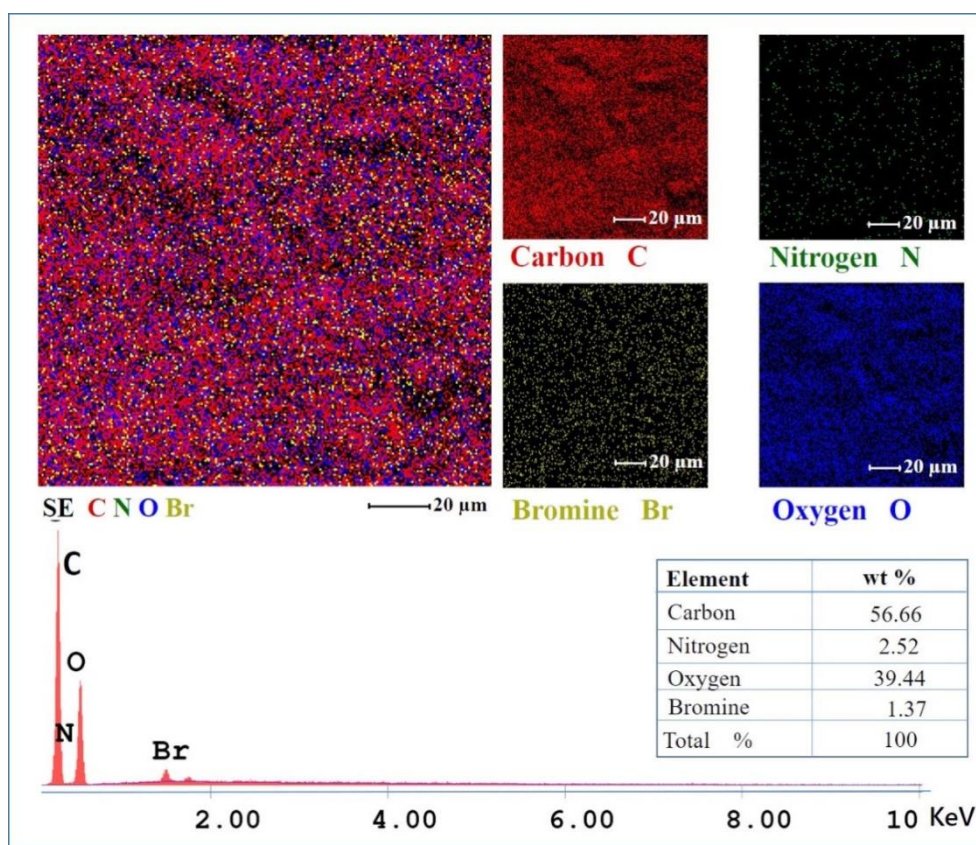


Figure 6.11: EDX Mapping and EDX spectrum of P(LA_{0.95}-ADTC_{0.05}-QAS-12C), 5000× magnification.

6.1.4. Antimicrobial Assessment of Polymers

As described in the experimental section, 96-well plates were used to determine the antimicrobial activities of the materials. First, the antimicrobial activity of ammonium salts of different alkyl chain length (propyl, hexyl and dodecyl) were determined against Gram-positive (*S. aureus*) bacteria. The concentrations of the salts for the experiment were adjusted according to the concentration in the copolymer (containing 30 mol% of ADTC) and the results of antimicrobial activity against *S. aureus* are shown in Figure 6.12. The quaternary ammonium salts with dodecyl group (QAS-12C) exhibited a strong antimicrobial activity compared with control (no compound) while the smaller groups had no significant effects on bacteria. In the next step, on-contact antimicrobial activity against *S. aureus* of the copolymer containing 30 mol% of ADTC was investigated after QAS-12C, QAS-6C and QAS-3C, respectively, were introduced. These copolymers dissolved in 100% methanol. For this reason, wells coated only with methanol were also added to the following experiments (Figure 6.13).

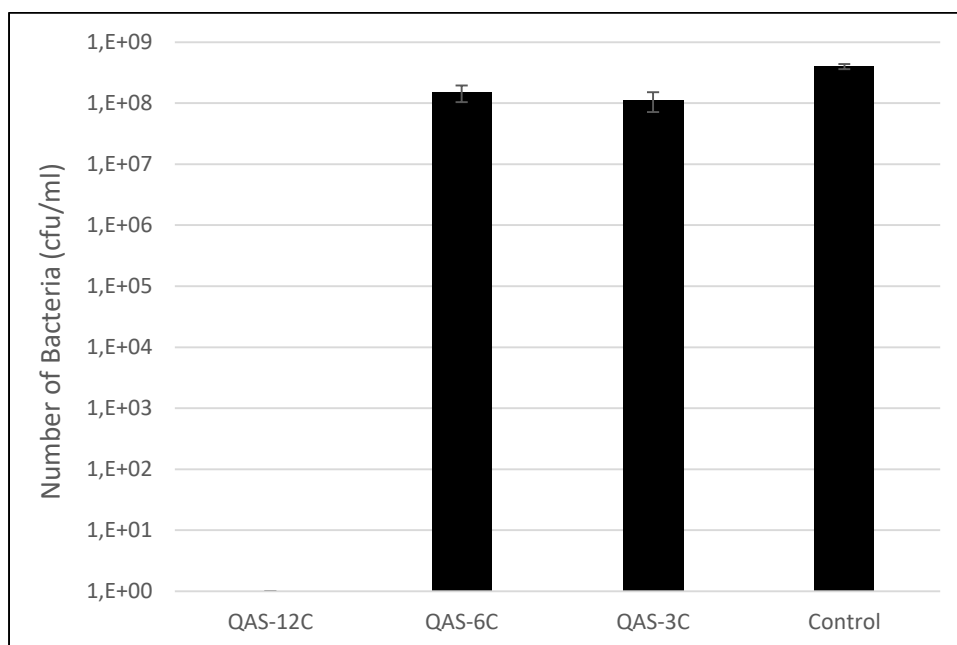


Figure 6.12: Antimicrobial activity results against *S. aureus* of quaternary ammonium salts substituted with dodecyl, hexyl, propyl groups over 24 h (QAS-3C, QAS-6C and QAS-12C refer to *N,N*-dimethyl-*N*-prop-2-yn-1-yl(prop-2-yn-1-yl(dodecane)-1-ammonium bromide).

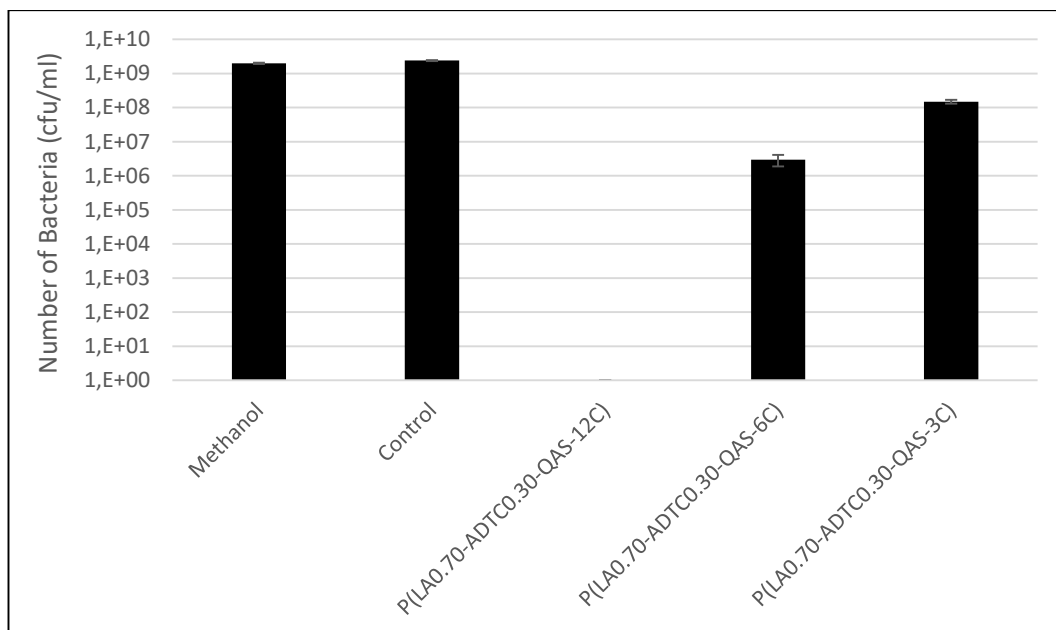


Figure 6.13: Antimicrobial activity results against *S. aureus* of methanol and copolymers after quaternary ammonium salts substituted with dodecyl (QAS-12C), hexyl (QAS-6C) and propyl (QAS-3C) carbon length were introduced into the P(LA_{0.70}-co-ADTC_{0.30}) backbone by click chemistry.

According to the results, the copolymer with the dodecyl group in the quaternary ammonium salt exhibited strong antimicrobial activity and no bacterial growth on agar plates was observed after incubation at 37°C for 24 h (Figures 6.13 and 6.14). We also checked if there was any leaching from the polymer to the environment. For this purpose, the coating polymer (P(LA_{0.70}-ADTC_{0.30}-QAS-12C)) was incubated with water. Then, the water was analysed using the US Environmental Protection Agency (EPA) Method 300.0 for bromide analysis. The results showed that there was insignificant leaching that will not affect the antimicrobial results (see Figure C1.1, Appendix). So, it can be said that polymer coatings have an on-contact killing property. According to Figures 6.13 and 6.14, the results support the studies conducted by Zhu *et al.* [29] who state that the length of the alkyl chain in the quaternary ammonium group affects the antimicrobial activity of the polymers. As the carbon chain of the alkyl group lengthened, the activity became stronger because of the increasing interaction between the groups and the lipid layer of the cell wall. Moreover, the results indicate that the antimicrobial activity of the polymeric form of the quaternary ammonium salt with hexyl carbon length is more active than the

monomeric form at the same concentration (Figures 6.13 and 6.14), consistent with the literature [13].

Finally, after obtaining the result that the copolymer with the dodecyl group in the quaternary ammonium moiety exhibited a strong antimicrobial activity, the experiments were carried out for other copolymers containing less than 30 mol% of the dodecyl group. It was found that all copolymers containing the dodecyl group were very effective against *S. aureus* and *E. coli*. No bacterial growth on the agar plates was observed after incubation at 37 °C for 24 h (Figures 6.15 and 6.16).

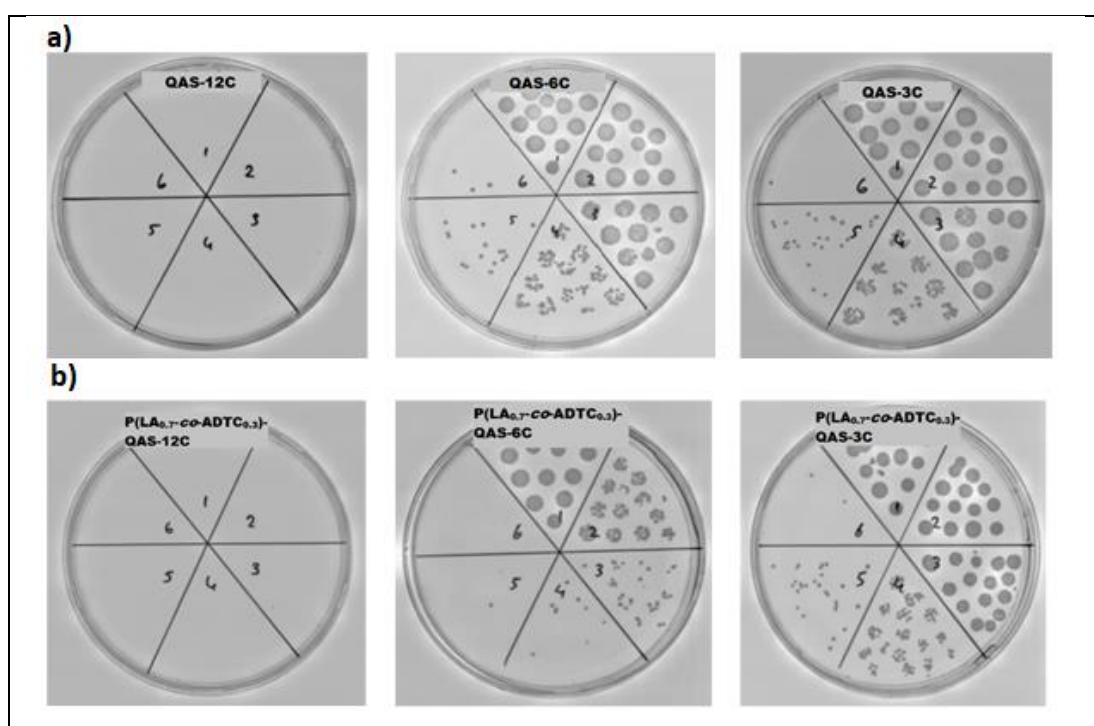


Figure 6.14: The results of antimicrobial activity against *S. aureus* on agar plates:
a) quaternary ammonium salts in the monomeric form, and b) copolymers containing the quaternary ammonium salts.

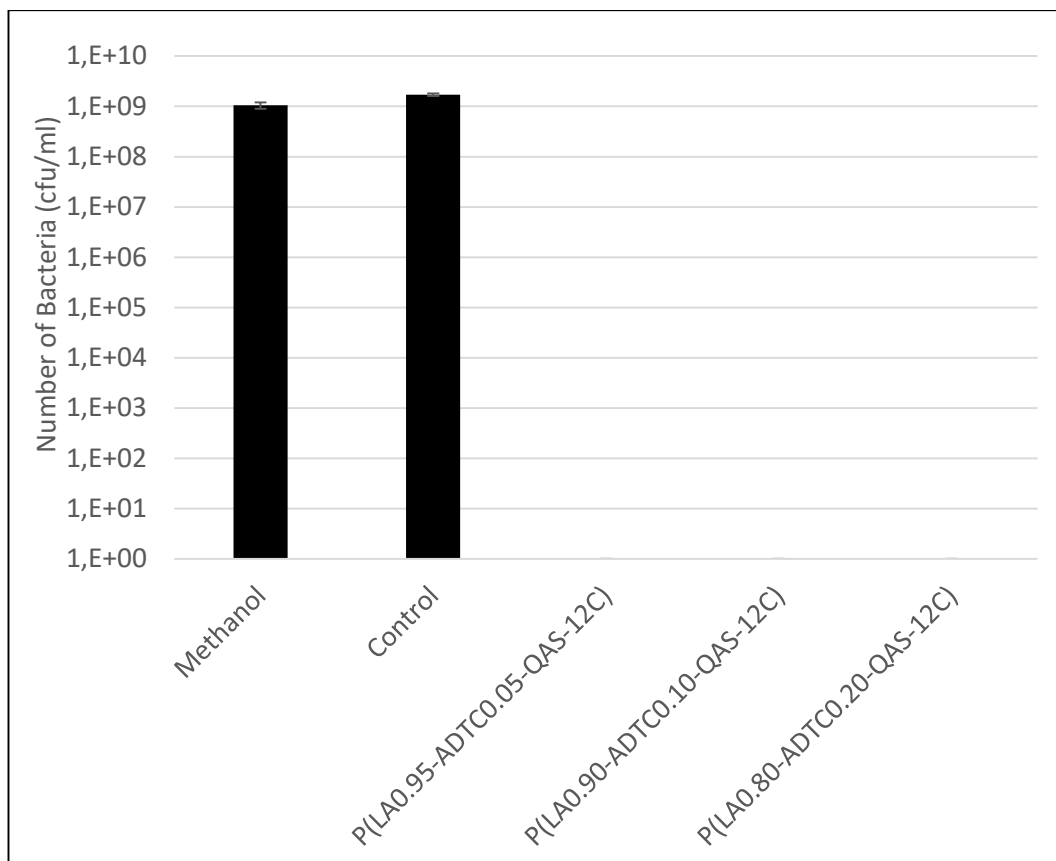


Figure 6.15: Antimicrobial activity results against *S. aureus* of copolymers containing 5, 10 and 20 mol% of quaternary ammonium salt substituted with dodecyl carbon length.

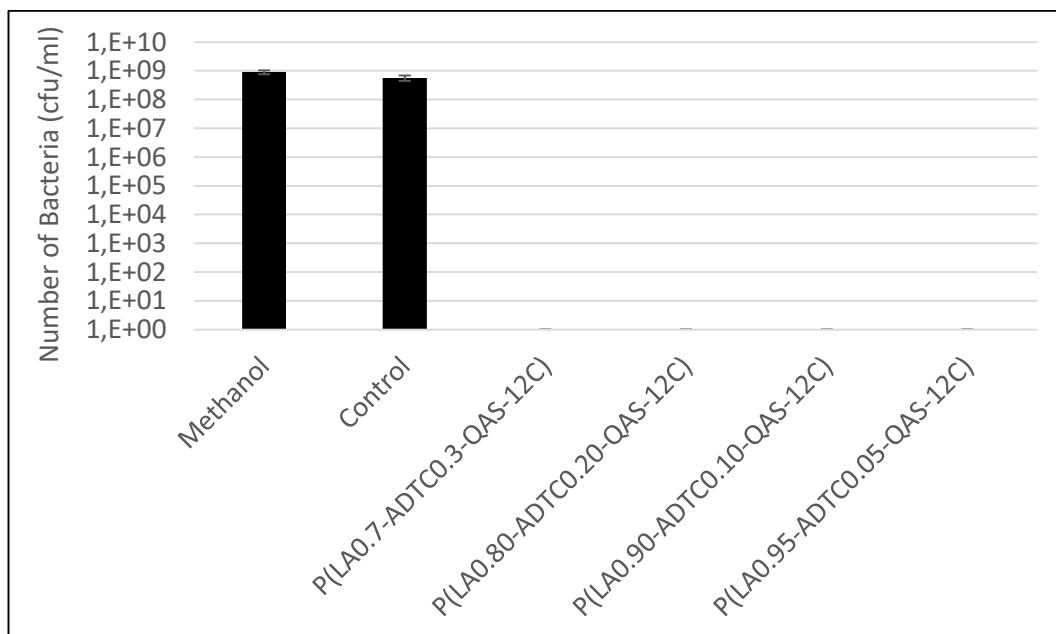


Figure 6.16: Antimicrobial activity results against *E. coli* of copolymers containing 5, 10 and 20 mol% of quaternary ammonium salt substituted with dodecyl carbon length.

6.2. Preparation of Antimicrobial PLA Film

6.2.1. FTIR Analysis

FTIR was performed to identify the existence of antimicrobial polymer (P2 shown in Figure 5.1) on the surface of PLA film. FTIR spectra of the reference PLA film and coated film (PLA+25) are illustrated in Figure 6.17. As explained in section 6.1.3, occurrence of an absorption peak at 1608 cm^{-1} for polymer P2, characteristic of the vibration of the unsaturated triazoles, confirmed a successful click reaction. The FTIR spectra of the PLA+25 film showed presence of a band at 1608 cm^{-1} , too. Thus, the FTIR spectrum revealed that there were antimicrobial polymers on the PLA film.

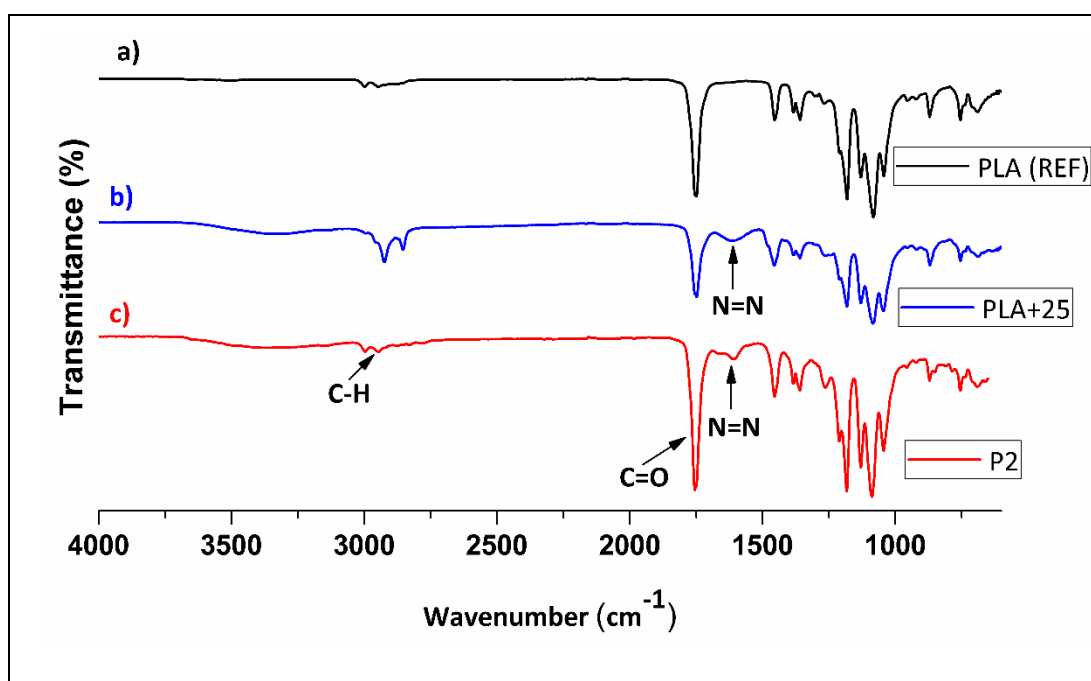


Figure 6.17: FTIR spectra of a) PLA (REF), b) PLA+25, and c) P2.

6.2.2. Antimicrobial Activity of PLA Films

The results of the antimicrobial tests performed against *S. Aureus* were presented in Figure 6.18. The antimicrobial activity of the coated films was examined through contact-killing test as explained in experimental part. The control film that there was not any antimicrobial polymer on surface did not inhibit bacterial growth.

The decrease in the $\log_{10}$ CFU/ml values indicated that PLA films sprayed 5 times and 15 times effectively inhibited the growth of microorganisms (Figure 6.18). After these results, the antimicrobial activity of the PLA film sprayed 25 times did not investigated.

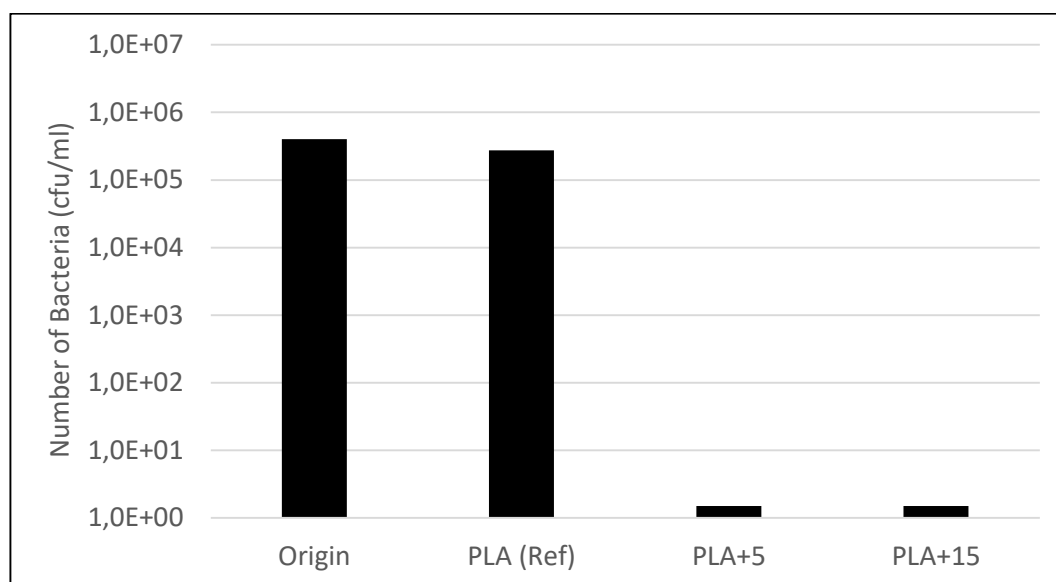


Figure 6.18: Antimicrobial activity results against *S. aureus* of reference film and coated films with polymer having antimicrobial property.

6.2.3. Mechanical Properties

Tensile test analyses were occurred to determine the values such as the elongation at break (%E), Young's modulus (E), and tensile strength (TS) etc. These values are important to obtain mechanical information of the polymers. It was known that films desired for food packaging applications needed acceptable flexibility to prevent breaking during the packaging procedure [93].

The mechanical properties of reference PLA film and coated films are shown in Figures 6.19 and 6.20. The values were also given in Table 6.2. Stress-strain curves (Figure 6.19) showed that PLA (Ref) was brittle while the other coated three films were ductile. Hence, it can be said that coating process contributed to the improvement of mechanical properties because of some plasticizing effect on film. However, it was seen that films were broken early as the coating ratio increased. Also, PLA+25 showed the lowest Young's modulus (E) while PLA (Ref) showed the highest

'E' value and 'E' was decreased by increasing coating ratio as shown in Figure 6.20 (a). In addition, a certain increase in %E for coated films, especially for PLA+5 and PLA+15, was observed Figure 6.20 (b). And, PLA+5 showed the highest TS (Figure 6.20 (c)). So, that can be said clearly the film with the lowest coating ratio is the most suitable in terms of mechanical properties.

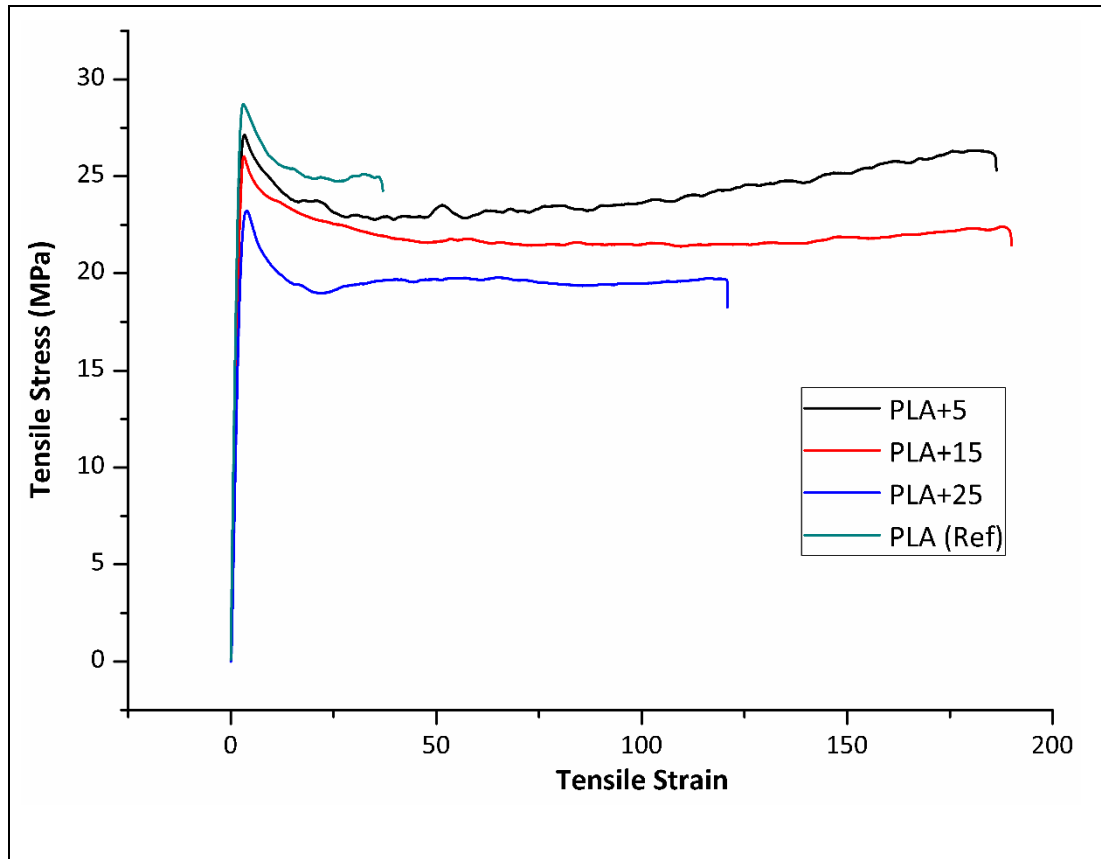
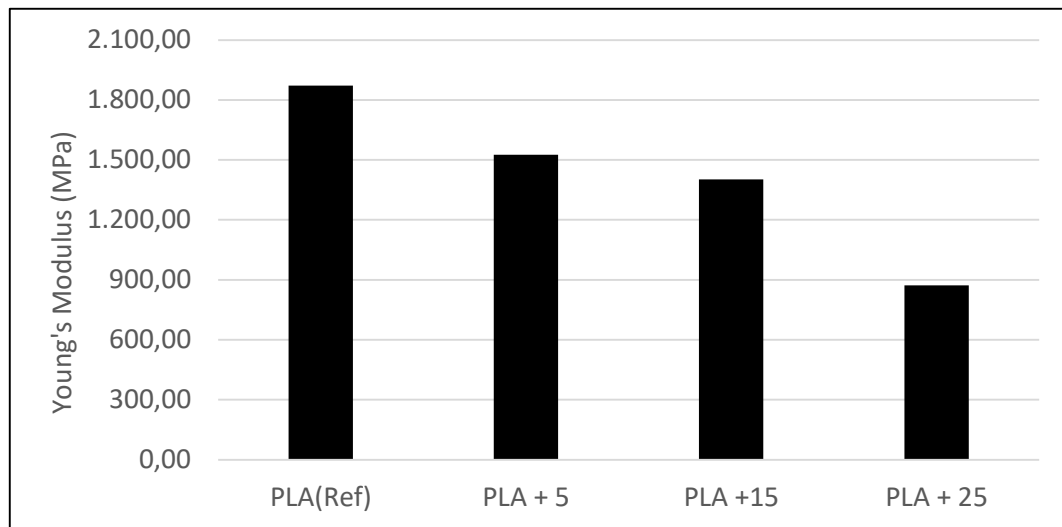
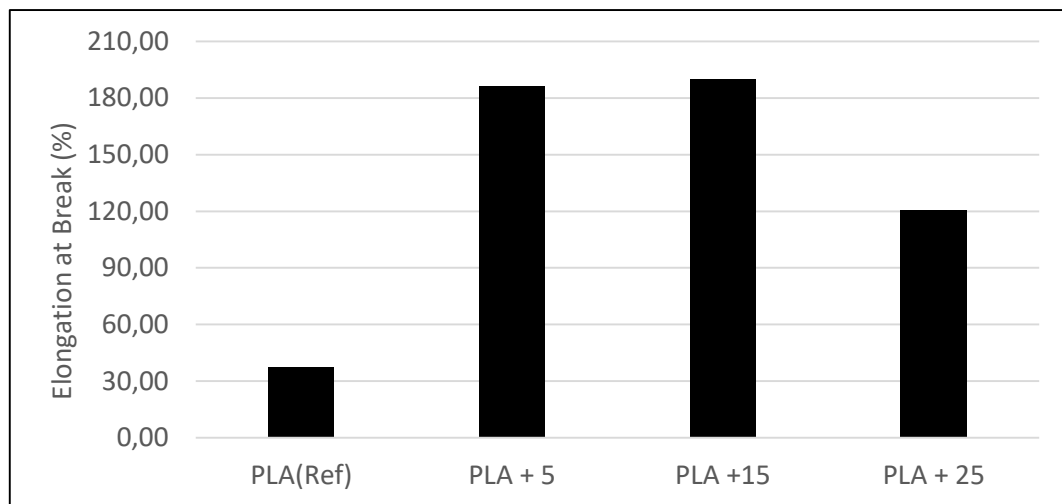


Figure 6.19: Tensile stress–strain behavior of PLA films.

a)



b)



c)

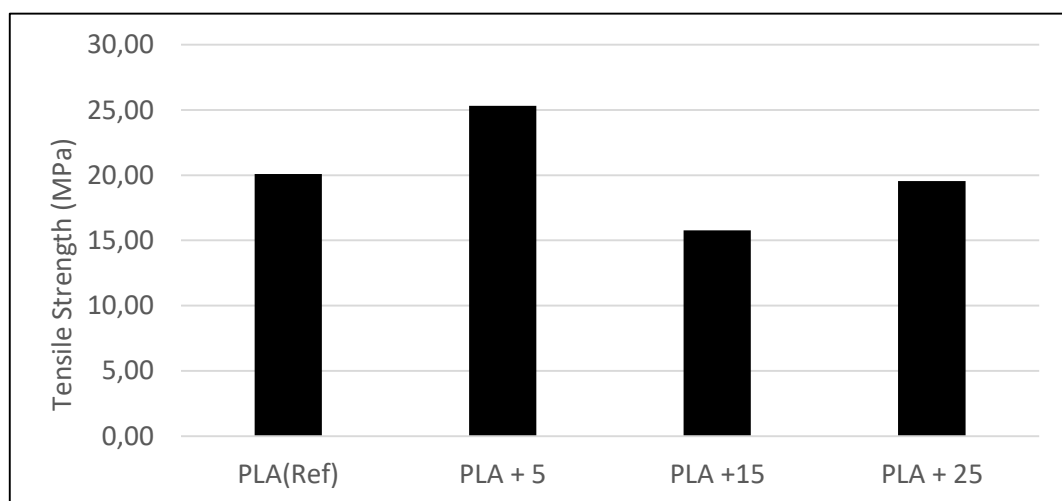


Figure 6.20: Mechanical properties of PLA films a) Young's modulus (MPa), b) Elongation at Break (%), and c) Tensile Strength (MPa).

6.2.4. Thermal Properties

Differential scanning calorimetry (DSC)

The thermal phase transition temperatures (T_m and T_g) of the polymers were investigated via DSC. DSC traces recorded during the second heating cycle of the film samples were shown in Figure 6.21. The glass transition temperature (T_g), and melting temperature (T_m) were summarized in Table 6.2. The peak T_g and T_m slightly decreased with the increase of coating ratio. It can even be said that T_g and T_m have not changed significantly. T_g of reference PLA film was reported to be 50.41 °C. As the amount of coating ratio increased, the T_g value decreased to 50.04°C for PLA+5 and to 47.73 °C for PLA+25 films. Besides, the peak of crystallization temperature (T_c) was more apparent for reference film (99.10 °C), and the degree of crystallinity (X_c) was calculated as 90.02 %.

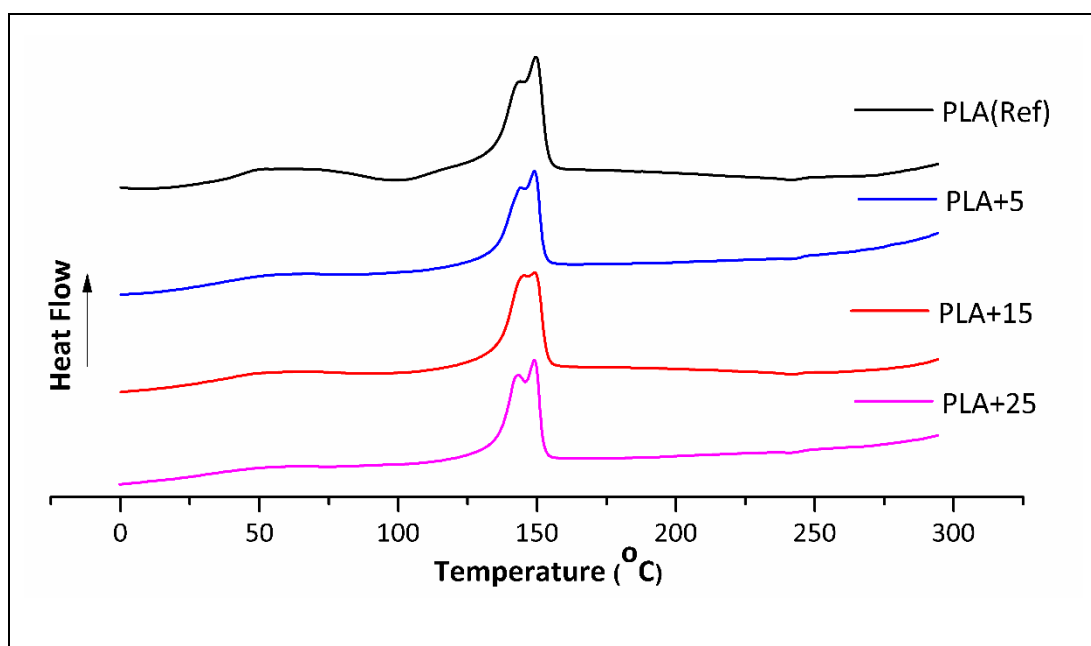


Figure 6.21: Differential scanning calorimetry thermograms of the PLA films.

These results are consistent with the results obtained from tensile tests. This reduction in T_g value is a good sign of the extent of the plasticizing effect caused by coating process.

Table 6.2: Mechanical, thermal and barrier properties of the PLA films.

	PLA (Ref)	PLA+5	PLA+15	PLA+25
Tensile Strength (MPa)	20.08	25.31	15.77	19.56
Elongation at Break (%)	37	186	190	120
Young's Modulus (MPa)	1872.63	1526.43	1402.46	871.99
T_g (°C) ^a	43.58	42.34	40.97	38.96
T_m (°C) ^b	149.72	149.09	149.02	149.01
$T_{d,onset}$ (°C) ^c	99.72	99.75	99.51	100.08
$T_{d,max}$ (°C) ^d	350.50	353.58	355.00	352.16
Char Yield (%) ^e	0.09	2.19	2.46	2.15
Oxygen Permeability (cm ³ .mm/m ² .day.atm)	39.55	n.c. ^f	n.c. ^f	42.00
Water Vapor Permeability x10 ⁻¹³ (g·m/m ² ·s·Pa)	5.24	n.c. ^f	n.c. ^f	5.86
^{a,b} Determined by DSC with a heating rate of 10 °Cmin ⁻¹ under nitrogen flow. ^c $T_{d,onset}$ denotes the onset decomposition temperature of the polymers. ^d $T_{d,max}$ designates the temperature that corresponds to the maximum rate of weight loss. ^e The percentage weight remaining at 700 °C. ^f Not calculated.				

Thermogravimetric analysis

The thermal stability of all the PLA film samples was investigated by TGA under a nitrogen atmosphere at a heating rate of 10 °C min⁻¹ from room temperature to 700 °C. The data related to their onset ($T_{d,onset}$) and maximum thermal decomposition ($T_{d,max}$) temperatures as well as their percentage char yields are summarized in Table 6.2. Also, thermal degradation of all samples was shown in Figure 6.22.

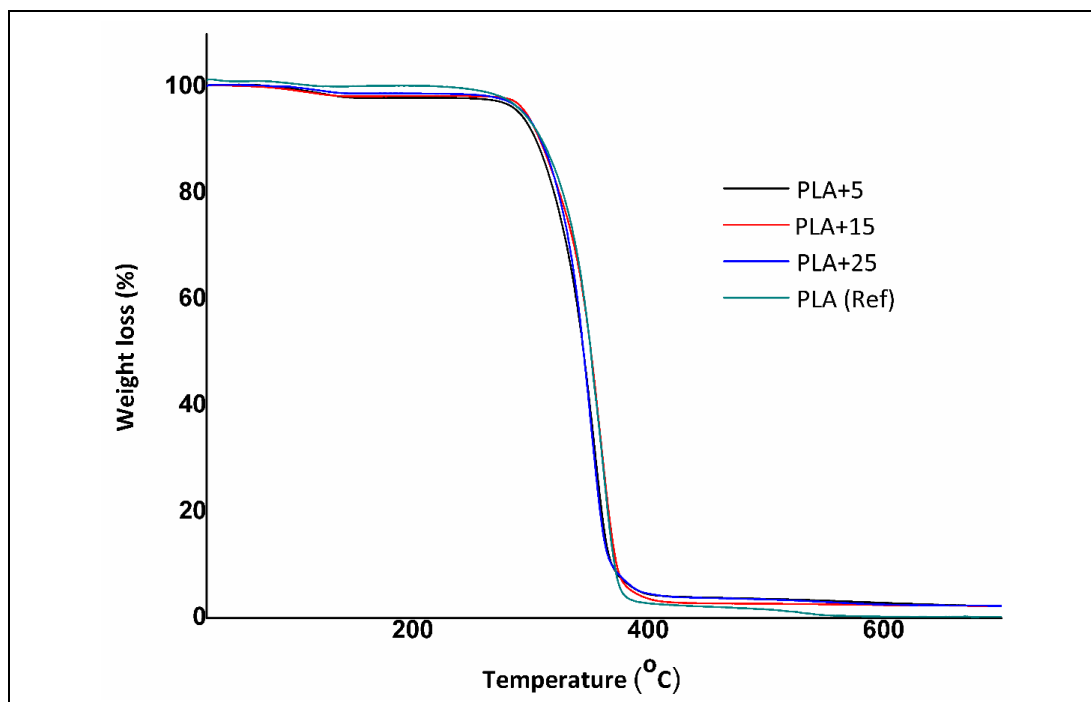


Figure 6.22: Thermogravimetric analysis curves of the PLA films.

According to the TGA results, the percentage char yields of the films were increased slightly after the coating process was applied on the reference PLA film. But, the increase in coating ratio did not change the values of char yields significantly. In addition, it was observed that $T_{d,onset}$ and $T_{d,max}$ values of all film samples did not change dramatically, too. As a result, it can be clearly stated that the thermal stability of PLA films was not significantly affected by the coating process.

6.2.5. Barrier Properties

Water Vapor Permeability (WVP)

One of the most basic features of food packaging is to prevent or minimize moisture transfer between the food and the surrounding atmosphere [89], [93]. The effect of coating process on WVP of PLA films was shown in Table 6.2. In this study, the WVP of PLA films was not significantly affected by the coating with antimicrobial polymer in the ratio of 25 times compared to PLA reference film. Coating of antimicrobial polymer onto PLA reference film increased the WVP from 5.24×10^{-13} g·m/m²·s·Pa (PLA(Ref)) to 5.86×10^{-13} (g·m/m²·s·Pa) (PLA+25) (Figure 6.23). The decrease in water vapor barrier properties is the result of the decrease in Tg values.

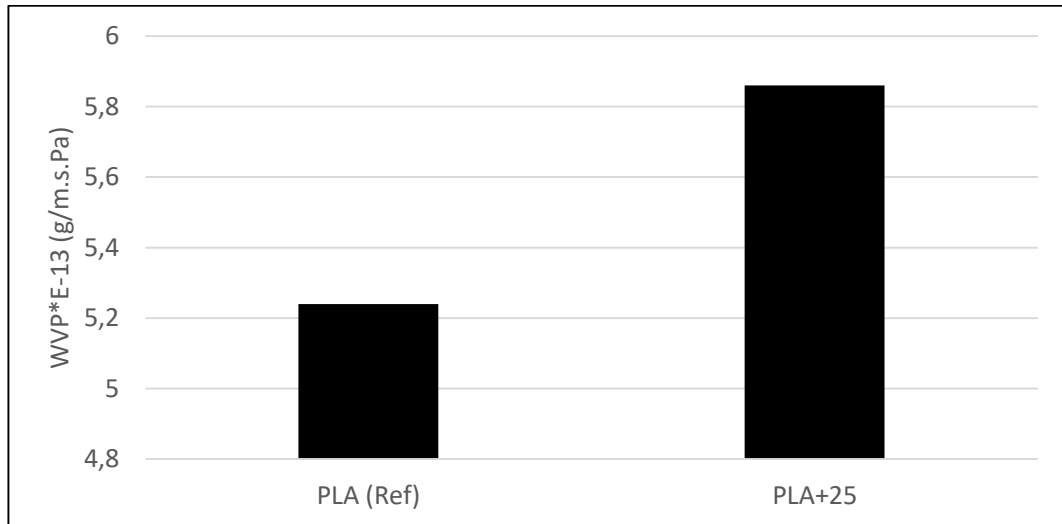


Figure 6.23: Effect of coating process on WVP of PLA films.

Oxygen permeability (OP)

The oxygen permeability (OP) values of the PLA films is shown in Figure 6.24. There were no significant differences in oxygen permeability (OP) between two PLA films (Table 6.2). Similar to the results of the water vapor permeability test, the coating process had no significant effect on the oxygen permeability values. Coating of antimicrobial polymer onto PLA reference film increased the OP from 39.55 ($\text{cm}^3 \cdot \text{mm} / \text{m}^2 \cdot \text{day} \cdot \text{atm}$) (PLA(Ref)) to 42 ($\text{cm}^3 \cdot \text{mm} / \text{m}^2 \cdot \text{day} \cdot \text{atm}$) (PLA+25) (Figure 6.24).

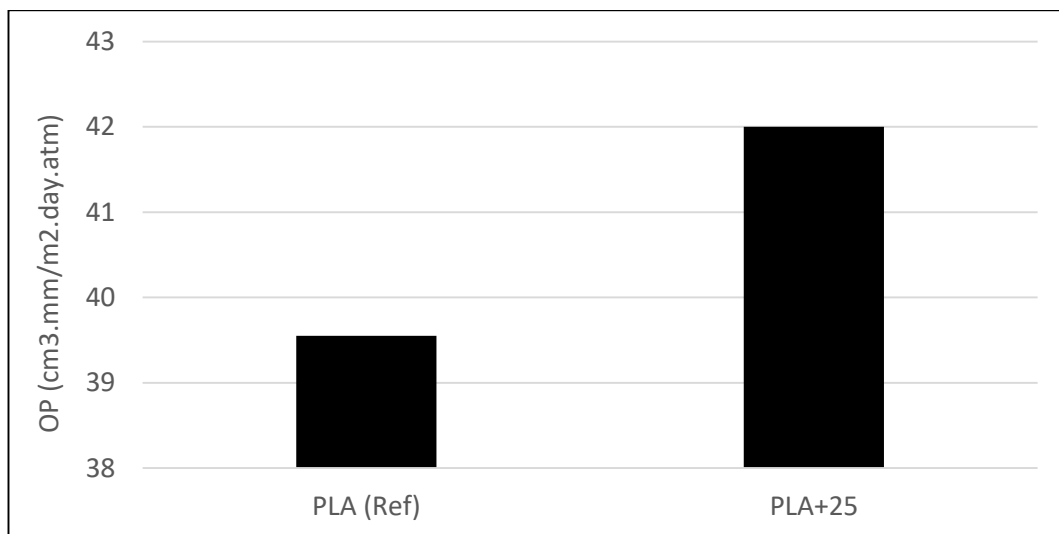


Figure 6.24: Effect of coating process on OP of PLA films.

6.2.6. Scanning Electron Microscopy Analysis

The SEM microphotographs of PLA films were shown in Figures 6.25-27. Figure 6.25 represents SEM micrographs of all PLA films at 200 microns scale bar. Lamellar structures were obtained on the PLA+5 and PLA+15 films as seen in that figure. However, these structures were not observed on PLA+25 and PLA (Ref) films. The 5 and 15 times coating process caused crystallization on the PLA film surfaces. But, coating process lost crystallization effect on PLA films as the coating ratio increased. It was thought to be due to diffusion of antimicrobial polymers dissolved in acetone into the neat PLA film.

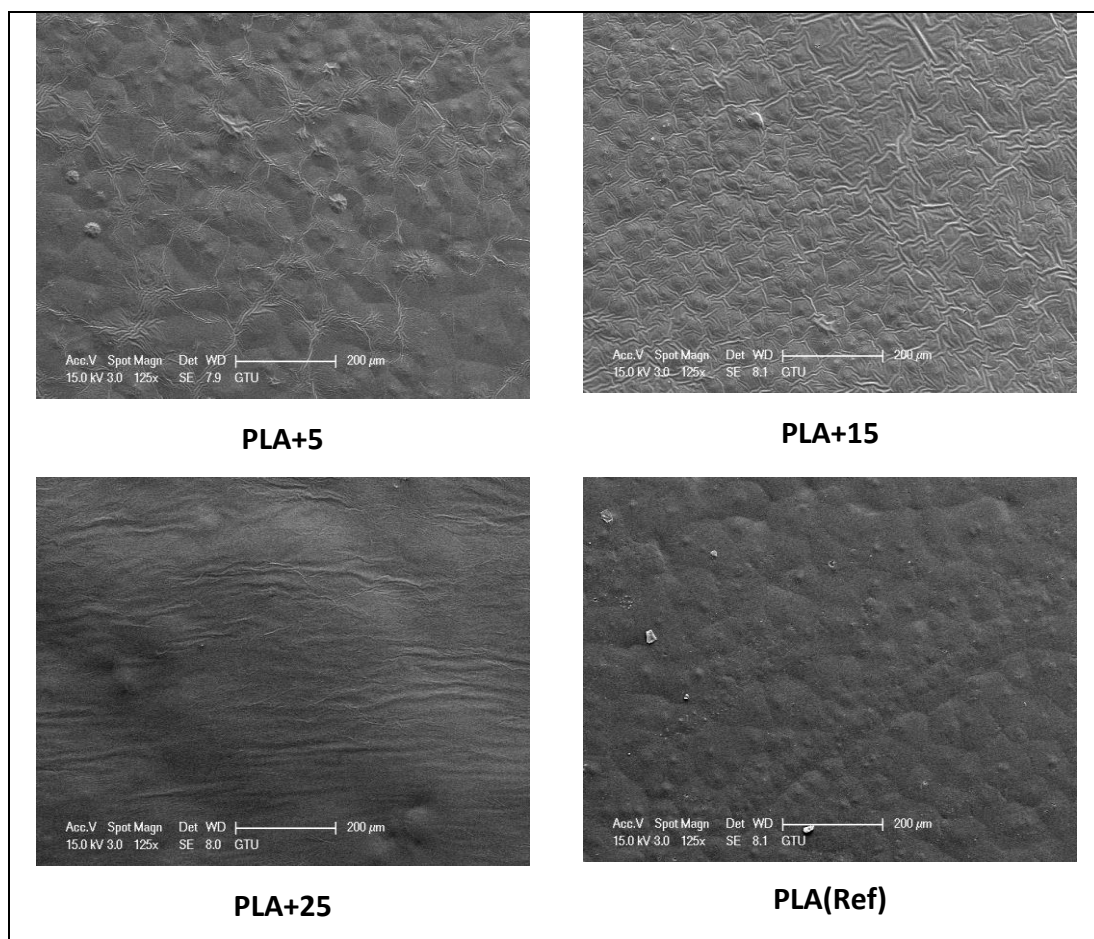


Figure 6.25: SEM micrographs of all PLA films (Magnification: X125, scale bar: 200 microns).

The SEM microphotographs of PLA+25 film were shown in Figure 6.26 at different scale bars. No voids or pits were observed on the surface of the film at 200

microns scale, but drops of approximately 2-2.5 microns in diameter were observed in a 10 micron sized bar. It proved that the antimicrobial polymer solution that employed for the coating of the PLA film could constitute a homogenous structure on the surface.

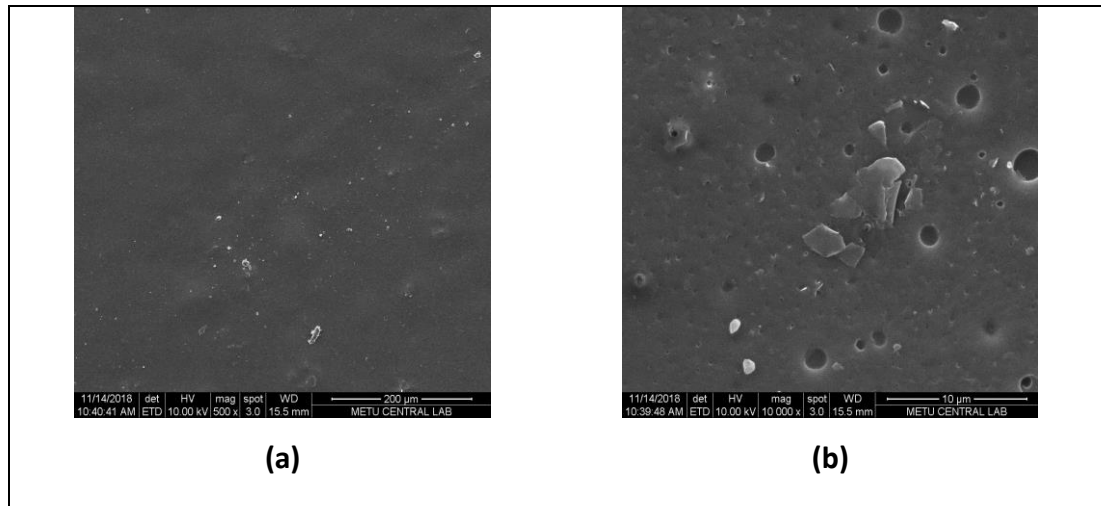


Figure 6.26: SEM micrographs of PLA+25 film a) Magnification: X500, scale bar: 200 microns, b) Magnification: X10 000, scale bar: 10 microns.

Figure 6.27 shows the scanning electron micrograph of impact fractured surface of PLA+25 at different scale bars. It was seen that, the antimicrobial polymer was penetrated into the film up to 100-150 microns thick.

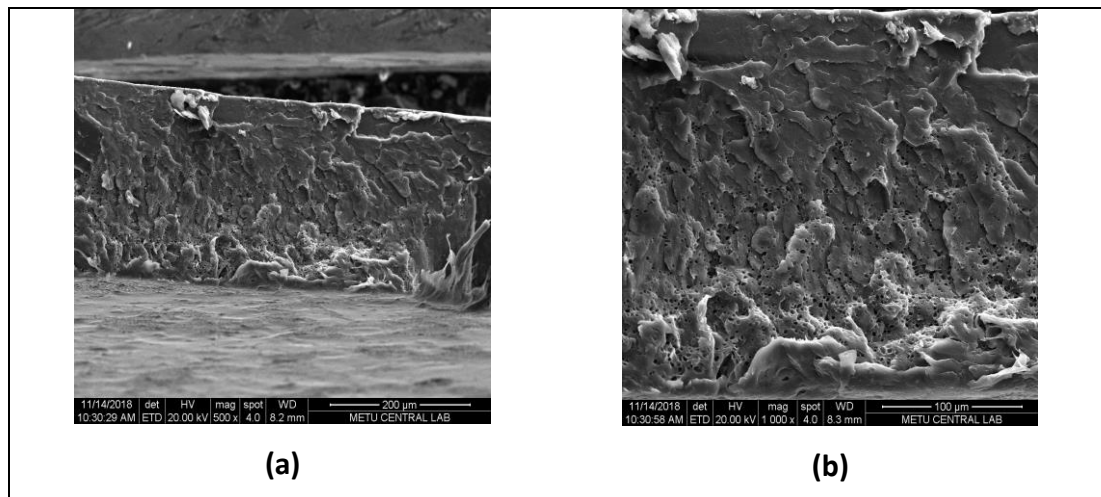


Figure 6.27: SEM micrographs of impact fractured surface of PLA+25 film a) Magnification: X500, scale bar: 200 microns, b) Magnification: X1 000, scale bar: 100 microns.

7. CONCLUSION

This study was carried out in two stages. In the first part, biodegradable poly(lactic acid) based copolymers bearing pendant antimicrobial agent groups were successfully fabricated with a combination of ring-opening copolymerization and copper(I)-catalysed azide–alkyne cycloaddition click reaction in a two-step reaction procedure. First of all, biodegradable PLA copolymers bearing azido groups were synthesized by the ring-opening copolymerization of L-lactide and 2,2-bis(azidomethyl)trimethylene carbonate in the presence of 1-dodecanol as protic co-initiator and tin(II) 2-ethylhexanoate ($\text{Sn}(\text{Oct})_2$) as the catalyst. Then, alkyne functionalized quaternary ammonium salts were attached onto the azido groups of the copolymers through a Huisgen 1,3-dipolar cycloaddition reaction to give PLA antimicrobial activity. The chemical structure and composition of the copolymers were clearly confirmed using ^1H -NMR, FT-IR and GPC. Thermal phase transition temperatures (T_m and T_g) and the thermal stability of the polymers were investigated by DSC and TGA experiments, respectively. The antimicrobial activity tests were carried out against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria by the drop plate method. Although similar studies on polycaprolactone (PCL) have been reported in the literature, to the best of our knowledge this is the first report of the synthesis of PLA to impart antimicrobial behaviour with antimicrobial agents attached covalently. So, the antimicrobial agents will not diffuse and this provides many opportunities for the use of PLA in different applications from biobased materials to the food industry.

As mentioned before, lactide copolymers are more hydrolytically sensitive than caprolactones and in this study it was possible to apply click chemistry without backbone degradation of the polymers. The biocidal effect of PLA grafted with ammonium cations was investigated by the drop plate method. The length of the substituted alkyl chain in the quaternary ammonium group affects the antimicrobial abilities of the polymers. The quaternary ammonium salts substituted by dodecyl groups exhibited high antimicrobial activity against Gram-negative and Gram-positive bacteria both in monomeric and polymeric form, even with a very low compositional ratio.

In the second part, the polymer containing the largest amount of antimicrobial group (P(LA_{0.7}-co-ADTC_{0.3}-QAS-12C), P2) was employed in the preparation of antimicrobial PLA films via spray coating method. This antimicrobial polymer was dissolved in acetone with the ratio of 5 % (wt %). On the other hand, neat PLA films (PLA/PEG, 90/10) were prepared by solvent casting method. Then, the one side of these films were coated in ratio of 5, 15 and 25 times with antimicrobial polymer dissolved in acetone, respectively. The antimicrobial activity tests of the films were carried out against Gram-positive (*Staphylococcus aureus*) bacteria by contact-active method. The effect of coated process on thermal properties are examined by TGA and DCS experiments. Also, the mechanical and barrier properties of the reference PLA and the most spray coated (PLA+25) films are investigated. As a result of analyses, it was observed that the coating process did not alter the thermal and mechanical/barrier properties of the PLA based films dramatically. So, that can be said clearly the least coated film, PLA+5, can answer the purpose. This situation significantly reduces the amount of chemicals to be applied, which provides crucial advantages both in terms of health and economy.

Consequently, PLA based films having antimicrobial activity on one side were obtained using a method different from the literature. The obtained films showed highly effective antimicrobial activity against *S. Aureus*. The use of polymers as antimicrobial agent offers some advantages because they are stable and non-volatile. Hence, they do not diffuse to the surrounding medium causing an environmental contamination, and also not permeate through skin such as small organic molecules, salts, metals and silver or copper. The obtained PLA based antimicrobial polymers are thought to have potential to be used in broader fields of applications such as food packaging, biomedical devices and implants.

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BIOGRAPHY

Figen Aynalı was born in 1986, İstanbul. She received his B.Sc. degree from Department of Chemical Engineering, Cumhuriyet University in 2010. In 2011, she started her M.Sc. study at the Department of Chemical Engineering, Marmara University under the supervision of Assoc. Prof. Gökçen Alev Altun Çiftçioğlu. Since 2014, being as a research assistant, she pursued her Ph.D. study under the supervision of Prof. Dr. Hasan Sadıkoğlu at the Department of Chemical Engineering, Institute of Natural and Applied Sciences, Gebze Technical University.

APPENDICES

Appendix A: Publications

Aynalı F., Doğancı E., Kayabaşı F., Şahin Z. M., Yılmaz F., (2015), “Laktid Monomerinin Halka Açılma Polimerizasyonunda Farklı Katalizörlerin Etkisinin Araştırılması”, 27. Ulusal Kimya Kongresi, Çanakkale, Türkiye, 23-28 Ağustos.

Doganci Dandan M., Aynali F., Doganci E., Yilmaz F., Ozkoc G., (2016), “Improving Mechanical and Thermal Properties of Poly(Lactic Acid) By Using POSS Cored Star-Shaped Poly(ϵ -Caprolactone) Polymers”, 46th IUPAC World Polymer Congress (MACRO 2016), Istanbul, Turkey, 17-21 July.

Aynalı F., Bayır S., Doğancı E., 2017, “Dansil Yan Gruplarına Sahip Biyobozunur Poli(Laktik Asit) (PLA) Sentezi”, 29. Ulusal Kimya Kongresi, Ankara ODTÜ-KKM, Türkiye, 10-17 Eylül.

Aynalı F., Doğancı E., Doruk T., Sadıkoğlu H., 2018, “Synthesis and characterization of antimicrobial polylactide via ring-opening polymerization and click chemistry methods”, Polymer International, 68, 385–393.

Aynalı F., Doğancı E., Doruk T., Özkoç G., Sadıkoğlu H., 2019, “Antimikrobiyal Polilaktik Asit Filmlerin Hazırlanması Ve Termal/Mekanik Özelliklerinin İncelenmesi”, 31. Ulusal Kimya Kongresi, Yıldız Teknik Üniversitesi, İstanbul, Türkiye, 10-13 Eylül.

Doganci Dandan M., Aynali F., Doganci E., Ozkoc G., 2019, “Mechanical, thermal and morphological properties of poly(lactic acid) by using star-shaped poly(ϵ -caprolactone) with POSS core”, European Polymer Journal 121, 109316.

Appendix B: C-NMR spectrum of some synthesized compounds

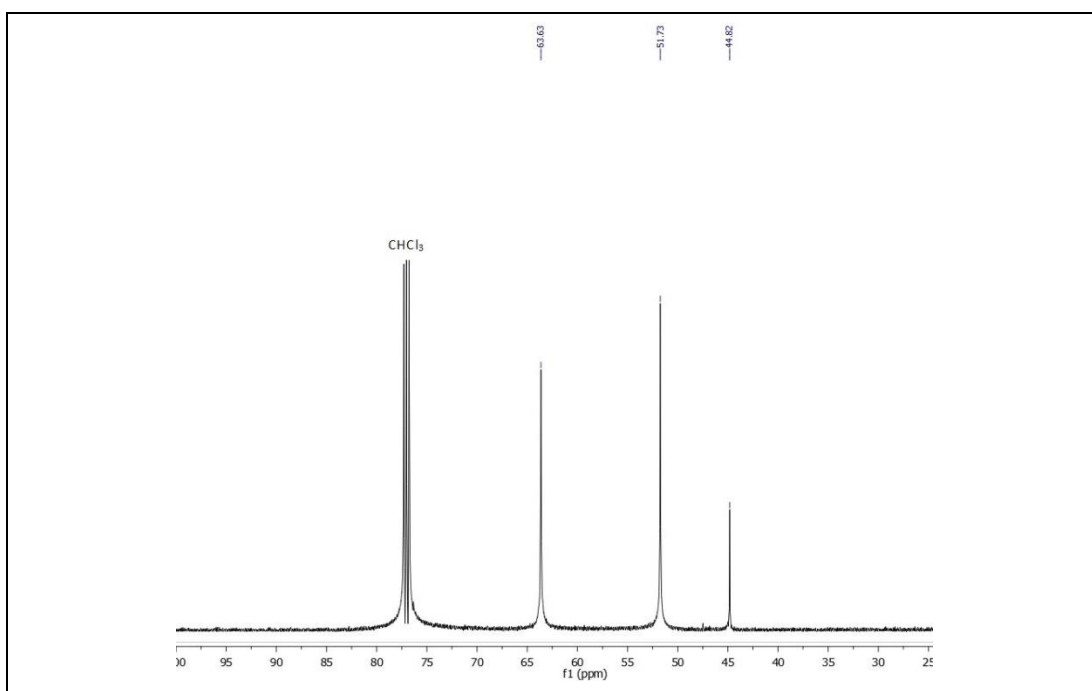


Figure B1.1: ^{13}C NMR spectra of 2,2-bis(azidomethyl)propane-1,3-diol.

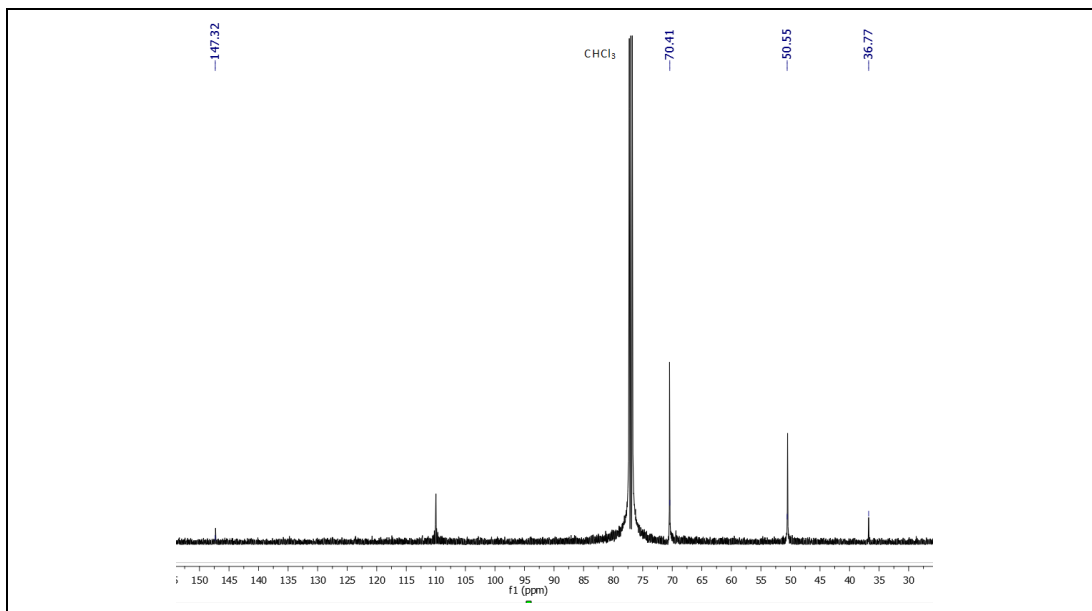


Figure B1.2: ^{13}C NMR spectra of azidocarbonatemonomer 2,2-bis(azidomethyl)trimethylenecarbonate (ADTC).

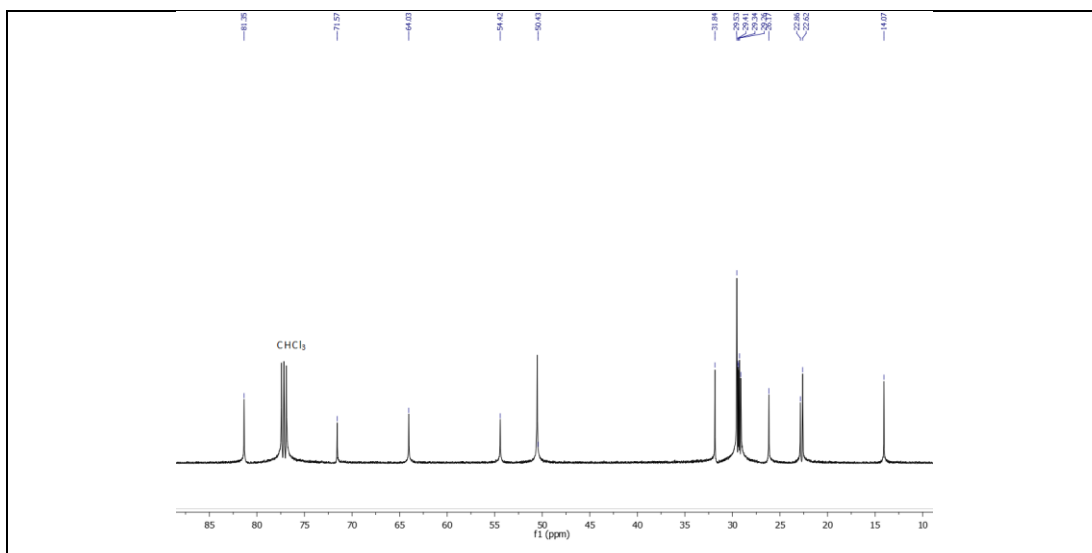


Figure B1.3: ^{13}C NMR spectra of N,N-dimethyl-N-prop-2-yn-1-yl-dodecane-1-ammonium bromide

Appendix C:

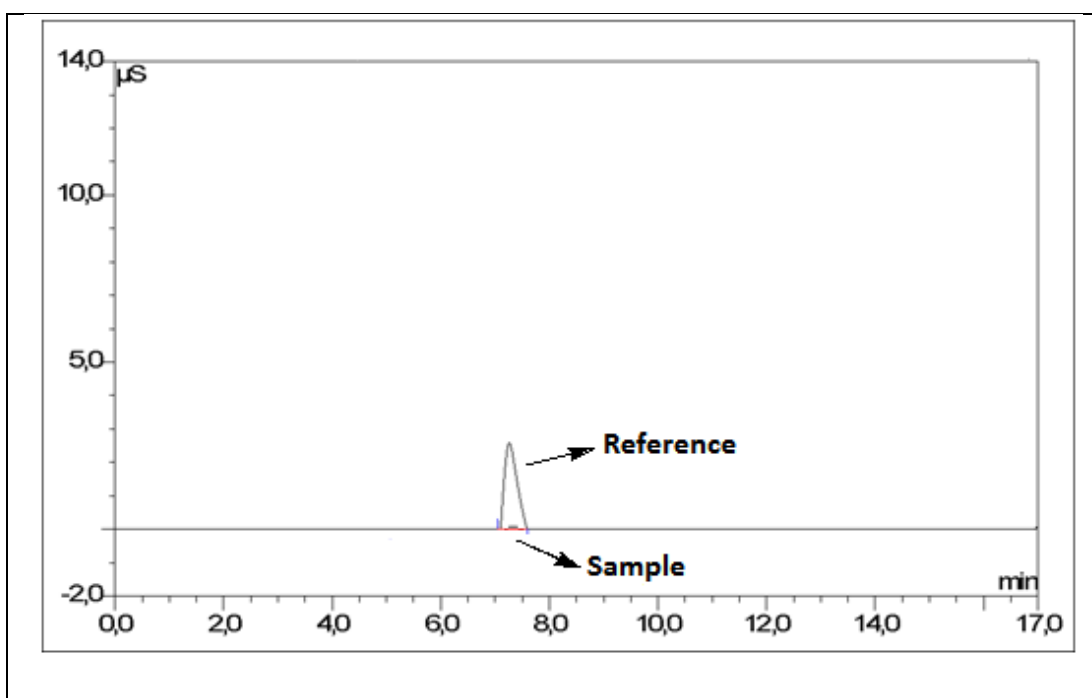


Figure C1.1: Chromatogram of Sample (P(LA_{0.70}-ADTC_{0.30}-QAS-12C)) and Reference (Copper (II) Bromide).