European Polymer Journal 212 (2024) 113056

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Preparation of caffeic acid grafted poly(D,L-lactide)-*b*-poly(2-hydroxyethyl methacrylate)/polylactide films with bioactive properties

Gamze Düz [a,b], Sinem Sipahioğlu Kara [c], Özlem Yılmaz [d], Muhammet U. Kahveci [b,*]

[a] *Istanbul Technical University, Graduate School, Polymer Science and Technology Program, Maslak, Sariyer, 34469 Istanbul, Türkiye*
[b] *Istanbul Technical University, Faculty of Science and Letters, Department of Chemistry, Maslak, Sariyer, 34469 Istanbul, Türkiye*
[c] *Bilecik Seyh Edebali University, Vocational School of Health Services, Department of Medical Services and Techniques, Bilecik, Türkiye*
[d] *Altıparmak Food Industry and Trade Inc., Balparmak R&D Center, Cavusbasi Cad., Cekmekoy, 34782 Istanbul, Türkiye*

ARTICLE INFO

Keywords:
Biopolymers
Poly(lactic acid)
Functional polymers
Antimicrobial film
Antioxidant film
Non-migratory additive

ABSTRACT

Derived from renewable sources like corn starch or sugarcane, poly(lactic acid) (PLA) presents an eco-friendly alternative to traditional petroleum-based plastics. PLA lacks inherent functional groups, posing a challenge for certain applications. The modification and functionalization of PLA contribute to the facilitation of innovative applications across diverse fields. To address this limitation, in this study a bioactive compound, caffeic acid, strategically grafted the onto poly(D,L-lactide)-*b*-poly(2-hydroxyethyl methacrylate) block copolymer (PLA-*b*-PHEMA). The resulting caffeic acid grafted copolymer (PLA-*b*-PHEMA-*g*-CA) was characterized by size exclusion chromatography, NMR, UV–Vis and FT-IR spectroscopies, and then blended with commercial PLA to produce films. The synthesis involves polymerizing 2-hydroxyethyl methacrylate with a poly(D,L-lactide) macroinitiator via ATRP method, yielding PLA-*b*-PHEMA. Subsequent functionalization via Steglich esterification yields PLA-*b*-PHEMA-*g*-CA. Characterization indicated a grafting ratio of 60.7%. Both grafted copolymer and films exhibited antioxidant property and antimicrobial effect against *S. aureus* and *E. coli*, showcasing potential applications in sustainable materials.

1. Introduction

In the context of growing environmental concerns and the imperative to adopt sustainable practices, the utilization of poly(lactic acid) (PLA) based materials has gained paramount importance in various industries. PLA, derived from renewable resources such as corn starch or sugarcane, represents a promising alternative to traditional petroleum-based plastics, contributing significantly to reducing carbon footprints. Petroleum-based polymers (polyethylene, polypropylene, poly(ethylene terephthalate), polystyrene, etc.) are widely used in various industries due to their mechanical performance, ease of processing and low cost [1]. Unfortunately, these conventional polymers are non-biodegradable and have significant impact on environment [2]. With the growing concern over environmental threats and ecological risks, there has been an increasing awareness of the need to reduce plastic pollution. Several waste managements are used to prevent and reduce the pollution such as restrictions, regulations, recycling and incinerating [1,3]. However, high-cost recycling process and low recycle rates have led to look for new materials that can be alternative to conventional plastics. In this context, biopolymers gained more research attention due to their biodegradable, nontoxic and renewable properties [2]. Biopolymers can be obtained directly from biomass, synthesized by microorganisms, or synthesized from bioderived monomer such as lactic acid [4]. PLA is one of the important biopolymer that can be polymerized from lactic acid and shows comparable physical properties to conventional plastics. Due to biodegradability and biocompatibility features, PLA is preferred to be used in many applications such as biomedical, packaging, textile etc. [5–9]. PLA as a packaging material has a wide range of forms as films, food containers, foams and coatings, and other types [10]. While PLA is an elegant sustainable, nontoxic, biodegradable polymer obtained from natural sources for food packaging materials, it is lack of chemical handle to be modified [10].

The pervasive use of conventional plastics in the packaging industry, which accounts for the largest share of global plastic consumption, has significantly contributed to plastic pollution; adopting biobased polymers derived from renewable sources is crucial for mitigating this environmental impact [11]. In 2020, 40.5 % of the world's total plastic production was used in packaging applications [12]. Biopolymers could

* Corresponding author.
E-mail address: kahvecimuh@itu.edu.tr (M.U. Kahveci).

https://doi.org/10.1016/j.eurpolymj.2024.113056
Received 29 February 2024; Received in revised form 16 April 2024; Accepted 19 April 2024
Available online 20 April 2024

be engineered not only to replace petroleum-based polymers in the food packaging industry but also to meet expectations for reducing food spoilage by enabling the development of bioactive food packaging materials. The Food and Agriculture Organization of the United Nations (FAO) reported that approximately one-third of global food production is lost or wasted annually, primarily due to factors such as the expiration of shelf life or deterioration induced by microbial activities [7]. As new technologies continue to emerge and the demand for extended shelf life of food products grows, material science approaches for reducing food waste have gained increasing attention [13]. There is tremendous effort for development of active packaging materials to increase its functionality in terms of protecting food [13–16]. Using biobased polymers instead of conventional polymers and preventing food spoilage through the use of bioactive materials would contribute to sustainability.

The active ingredients are effective in preventing oxidation when co-extruded with a packaging film, but they can diffuse from the packaging material to the food matrix [17]. Migratory active packaging systems pose a potential concern for regulatory authorities and consumers looking for natural ingredients. However, most active packaging technologies rely on a time-released migration of active compounds from the packaging material to the foodstuff [18]. Clean label term is referred to intend minimizing preservatives and stabilizers in food and the use of non-migratory packaging is one of the effective ways of this approach [19]. Unlike migratory active packaging based on the release of the active substance, non-migratory active packaging systems are used to prolong the shelf life of the food product without releasing a substance in food matrix [20]. Moreover, non-migratory active packaging systems could offer some potential advantages for regulatory acceptance and consumer demands. In these systems, the active substance is usually covalently bonded to the polymer surface, or the functionalized polymer with an active substance is used directly or by blending to form a film [21,22]. Phenolic compounds can be used in material science to improve the antioxidant or antimicrobial functionality of polymers. They can be integrated to polymer matrix by covalently, by non-covalently and by electrostatic interaction or hydrogen bonded [23–25]. 3,4-Dihydroxycinnamic acid, known as caffeic acid, is a cinnamic acid derivate phenolic which shows antimicrobial, antioxidant, antiviral, anti-inflammatory and antitumoral activities [26]. Caffeic acid has potential to enhance or increase the antioxidant and antimicrobial properties of materials when grafted onto polymer backbone.

In this study, we aimed to develop a well-defined and effective approach for preparation of functional polymers potentially to be used in active packaging. PLA is selected as parent material for its biodegradability, biocompatibility and sustainability. Since chemical modification of PLA is difficult due to lack of functional group on it, the copolymerization method with 2-hydroxyethyl methacrylate (HEMA) was chosen to graft the active substance on polymer chains. Caffeic acid was selected as the active compound due to its bioactive properties and its ability to undergo esterification reactions with hydroxyls through its carboxylic acid groups. Covalent bonding of caffeic acid to the hydroxyl units of PHEMA contributes to the non-migratory property of the grafted copolymer. The grafted copolymer was then blended with high-molecular-mass commercial PLA to prepare packaging films using the solvent casting method, which is simple and suitable for small-scale production.

2. Materials and methods

5-Amino-1-pentanol (Sigma), D,L-lactide (Acros Organics 99 %) tin (II) 2-ethylhexanoate ($Sn(Oct)_2$) (Sigma), α-bromoisobutyryl bromide (Sigma) triethylamine (TEA) (Sigma), sodium bicarbonate (Merck), magnesium sulphate (Merck), *N,N,N′,N″,N″*- pentamethyldiethylenetriamine (PMDETA) (Sigma), aluminum oxide (activated, neutral) (Sigma), 4-dimethylamino pyridine (DMAP) (Sigma), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC) (Sigma), caffeic acid (Sigma Aldrich), Nutrient Broth (Merck 1.05443), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 2592), Mueller Hinton Broth (Merck 1.10293), Mueller Hinton Agar (Merck 1.05437), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma- Aldrich), poly(ethylene glycol) (PEG, M_n: 1000 Da) (Sigma- Aldrich 8.07488) were used as supplied. Tetrahydrofuran (THF) (Sigma) was dried by refluxing over metallic sodium. Copper(I) bromide (Alfa Aesar) was purified by stirring in glacial acetic acid, filtration and rinsing with diethyl ether and ethanol, then dried under vacuum overnight. 2-Hydroxyethyl methacrylate (HEMA) (Sigma) was purified by passing over a short column of activated basic alumina.

Nuclear Magnetic Resonance spectroscopy (^{1}H NMR and ^{13}C NMR) measurements were conducted on an Agilent VNNMRS500 (500 MHz) in DMSO-d_6 and $CDCl_3$.

FT-IR measurements were performed with a Perkin-Elmer FTIR Spectrum One spectrometer. Spectra were collected with 6 scans in the range of 450 – 4000 cm^{-1}.

UV–Vis absorption spectra ranging from 250 to 800 nm were collected using a Peak Instruments C-7000 UV–Vis spectrophotometer. Antioxidant activities were analyzed using Thermo Scientific™ GENESYS™150 UV–Vis Spectrophotometer at 520 nm.

Gel permeation chromatography (GPC) measurement of PLA-Br was performed by TOSOH EcoSEC GPC system with refractive index and ultraviolet detectors. A TSK gel superhZ2000 (4.6 mm ID × 15 cm × 2 cm) column was used for separation and THF was used as eluent at a flow rate of 1.0 mL min^{-1} at 40 °C. Both detectors calibration was done with polystyrene standards having narrow molecular-weight distribution. Molecular weights of block copolymer and grafted copolymer were determined by Viscotek GPCmax (GPC) instrument equipped with an autosampler system consisting of a pump, three ViscoGEL GPC columns (G2000HHR, G3000HHR, and G4000HHR), a Viscotek differential refractive index detector. Dimethylformamide (DMF) was used as eluent at a flow rate of 1.0 mL min^{-1}. The number-average molecular weights were determined by using linear polystyrene standards.

2.1. Synthesis of caffeic acid grafted PLA-b-PHEMA polymer

2.1.1. Synthesis of 2-bromo-N-(5-hydroxypentyl)2-methylpropanamide (BNMP) initiator

Dual initiator for ROP and ATRP was prepared according to previously reported methods [27,28]. Briefly, 5-amino-1-pentanol (500 mg, 4.85 mmol, 3 eq.), triethylamine (TEA) (270 μL, 1.94 mmol, 1.2 eq) and 10 mL of THF were added into two-necked round bottom flask under nitrogen atmosphere. 2-Bromoisobutyryl bromide (200 μL, 1.61 mmol, 1 eq.) was added dropwise into reaction flask at 0 °C within 1 h. Reaction was carried out at room temperature overnight·THF was evaporated, residue was dissolved in ethyl acetate, transferred to separatory funnel and washed with 10 ml of 0.1 N HCl solution. Organic phase was separated and washed with saturated sodium bicarbonate solution. Organic layers of extracts were collected, then dried over magnesium sulphate and evaporated on a rotary evaporator. The initiator was dried under high vacuum (221 mg; yield: 54 %).

^{1}H NMR (500 MHz, $CDCl_3$, δ (ppm)) 6.78 (s, 1H, –CH_2-N*H*-), 3.66 (t, 2H, –CH_2-OH), 3.29 (q, 2H, –CH_2-NH-), 1.96 (s, 6H, C(−Br)(CH_3)$_2$), 1.59 (m, 4H, –CH_2-CH_2-CH_2–), 1.43 (m, 2H, CH_2-CH_2-CH_2–).

2.1.2. Synthesis of bromo end functional poly(D,L-lactide) (PLA-Br)

D,L-Lactide (6 g, 41.6 mmol, 72 eq.) and BNMP (0.145 g, 0.58 mmol, 1 eq., with minimal amount of toluene) were charged into 100 mL of two necked round bottom flask and catalytic amount of tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) was added under nitrogen atmosphere. Then flask was immersed in oil bath heated to 132 °C, stirred for 24 h. The reaction mixture was cooled to room temperature and dissolved with sufficient amount of dichloromethane (DCM). Precipitation of polymer was carried out in excess amount of methanol. The polymer was obtained after drying under vacuum for two days. (5.59 g; conversion: 93 %; $M_{n,theo}$ = 9,650 g/mol; $M_{n,NMR}$ = 12,850 g/mol).

^{1}H NMR (500 MHz, $CDCl_3$, δ (ppm)) 5.18 (m, 1H, –C*H*(CO)(CH_3), poly(lactide) chain); 4.31–4.42 (m, –OCH_2CH_2-); 1.95 (s, BrC(CH_3)$_2$); 1.57 (m, 3H, –CH(CO)(CH_3), poly(lactide) chain); 3.27 (q, –NH-CH_2-); 1.63–1.74 (m, –CH_2-CH_2-CH_2-); 1.3 (m, –CH_2-CH_2-CH_2-).

2.1.3. Synthesis of poly(D,L-lactide)-b-poly(2-hydroxyethyl methacrylate) (PLA-b-PHEMA)

Synthesis of the block copolymer was performed according to a previous study with slight modifications [27]. PMDETA (52 μL, 0.25 mmol, 1.6 eq.), CuBr (30 mg, 0.2 mmol, 1.3 eq.), HEMA (0.86 mL, 7.1 mmol, 46 eq.) and PLA (2 g, 1.6x10^{-4} mol, 1 eq.) were dissolved with 6 mL of DMSO in a 25-mL Schlenk tube, respectively, under nitrogen atmosphere at room temperature. Mixture was degassed in three cycled freeze–pump–thaw and immersed into oil bath at 80 °C. After 24-hour, reaction mixture was quenched by immersing into an ice bath. The resulting liquid mixture was diluted with THF and catalyst was removed by using neutral aluminum oxide column. After THF was evaporated, polymer was precipitated into excess amount of water, centrifuged and freeze dried for two days ($M_{n,theo}$ = 13,420 g/mol; $M_{n,NMR}$ = 17,700 g/mol) (See Supplementary Material for calculation of the theoretical molecular mass).

^{1}H NMR (500 MHz, DMSO-d_6, δ (ppm)) 5.45 (d, *H*OCH–(CH_3), poly(lactide) terminal unit); 5.17 (m, C*H*(CH_3); poly(lactide) chain); 4.79 (s, –COOCH_2CH_2O*H*); 4.20 (m, HOC*H*(CH3); poly(lactide) terminal unit); 3.89 (s,–COOC*H*$_2$CH_2OH); 3.57 (s,–COOCH_2C*H*$_2$OH); 1.60–1.70 (m, –C*H*$_2$-CH_2-C*H*$_2$-); 1.45 (m, CHC*H*$_3$, poly(lactide) chain); 1.28 (d, HOCH (C*H*$_3$)- poly(lactide) terminal unit); 0.77–0.94 (m, –C(C*H*$_3$) C*H*$_2$(COOCH_2CH_2OH)-).

2.1.4. Grafting caffeic acid onto PLA-b-PHEMA (PLA-b-PHEMA-g-CA)

Caffeic acid (220 mg, 1.22 mmol, 31 eq.), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (200 mg, 1.04 mmol, 26 eq.), 4-dimethylaminopyridine (DMAP) (180 mg, 0.15 mmol, 4 eq.) were added into a 25-mL Schlenk tube and dissolved in 10 mL of dry THF. The reaction mixture was vigorously stirred at 0 °C for 2 h. PLA-*b*-PHEMA copolymer (0.7 g, 3.95x10^{-5} mol, 1 eq.) was dissolved in THF, added to the Schlenk tube, and then reaction mixture was switched to room temperature slowly. After 24 h, the reaction mixture was filtered, precipitated into excess amount of water, centrifuged and freeze dried for two days. (grafting degree: 60.7 % confirmed by UV–Vis spectroscopy) ($M_{n,theo}$ = 16,270 g/mol; $M_{n,NMR}$ = 21,260 g/mol).

^{1}H NMR (500 MHz, DMSO-d_6, δ (ppm)) 6.55 – 8.58 (m, aromatic and olefinic protons of caffeic acid); 5.18 (m, C*H*(CH_3); poly(lactide) chain; 4.19 (m, HOC*H*(CH3); poly(lactide) terminal unit); 3.88 (s,–COOC*H*$_2$-CH_2OH); 3.55 (s,–COOCH_2C*H*$_2$OH); 1.62–1.98 (m, –C*H*$_2$C(CH_3)(COOCH_2CH_2OH)); 1.45 (m, 3 *J* = 7.0 Hz CHC*H*$_3$, poly(lactide) chain); 1.28 (d, HOCH(C*H*$_3$) poly(lactide) terminal unit); 0.77–0.94 (m, C(C*H*$_3$)(COOCH_2CH_2OH)).

2.2. Preparation of PLA-b-PHEMA-g-CA/PLA films

All film samples were prepared by solvent casting method. For this purpose, commercial high molecular mass PLA (Natureworks Ingeo 2500HP) [29] was dissolved in $CHCl_3$ by using magnetic stirrer and blended with PLA-*b*-PHEMA-*g*-CA polymer at different concentrations as given in Table 1 using homogenizer (Bandelin Sonopuls HD 2200). Overall, 0.75 g of each sample was dissolved in $CHCl_3$, poured onto petri dishes with 8 cm of diameter and dried in fume hood for 48 h at room temperature. PEG (5 % by mass) was physically incorporated into PLA films to enhance flexibility, mitigating the inherent brittleness of PLA while improving its processability.

Table 1
Composition of PLA films blended with PLA-*b*-PHEMA-*g*-CA by mass.

Sample	PLA (% by mass)	Poly(ethylene glycol) (% by mass)	PLA-*b*-PHEMA-*g*-CA % (PHC)
Neat PLA	100	0	0
PLA-0 % PHC	95	5	0
PLA-3 % PHC	92	5	3
PLA-5 % PHC	90	5	5
PLA-7 % PHC	88	5	7
PLA-10 % PHC	85	5	10

2.3. Bioactivity tests of grafted block copolymer (PLA-b-PHEMA-g-CA) and film samples (PLA-b-PHEMA-g-CA/PLA)

2.3.1. Antioxidant capacity of PLA-b-PHEMA-g-CA by DPPH• method

Briefly, DPPH• (0.60 mM) solution in methanol and sample solutions at different concentrations were mixed according to Table 2 and left in dark at room temperature. After 30 min, absorbance values were read at 517 nm. Methanol was used as blank and DPPH• solution without sample prepared immediately before readings was used for sample blank. Results were given as EC_{50} value (concentration that eliminates of 50 % DPPH• radical).

2.3.2. Antioxidant capacity of film samples by DPPH• method

The antioxidant capacities of film samples were conducted according to a previous study with slight modification [30]. Firstly, the film samples (1 cm × 1 cm) were soaked in 10 mL of DPPH solution (0.1 mM in ethanol) and incubated for 3 h in the dark at room temperature. For blank measurement, the same procedures were performed without a sample. Absorbances were read at 517 nm. The DPPH radical scavenging activity of the films was calculated according to Eq. (1).

$$Scavenging\ activity\ on\ DPPH\ radical\ (\%) = \left(1 - \frac{A_{sample}}{A_{control}}\right) x100 \qquad (1)$$

where $A_{control}$ and A_{sample} are the absorbance of the control (DPPH solution) and sample (DPPH of the sample), respectively. All measurements were performed in triplicate.

2.3.3. Antimicrobial activity of PLA-b-PHEMA-g-CA

Strains of the bacteria (*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* O157 ATCC 700728) used for the antimicrobial tests were obtained from the American Type Culture Collection. The *in vitro* inhibitory activities of extracts were investigated by the disc diffusion method. They were inoculated at nutrient agar at 37 °C for 24 h. The bacterial suspensions were adjusted to a concentration of 10^8 CFU/mL

Table 2
Concentrations of sample and volumes of solutions in antioxidant capacity assay.

Tube No	Final concentration of PLA-*b*-PHEMA-*g*-CA (μg/mL)*	Volume of methanol (μL)	Volume of sample solution (μL)	Volume of DPPH stock solution (μL)
0	0	1000	0	1000
1	2	960	40	1000
2	4	920	80	1000
3	6	880	120	1000
4	8	840	160	1000
5	10	800	200	1000
6	12	760	240	1000
7	14	720	280	1000
8	16	680	320	1000

against 0.5 Mc Farland. Sterilized discs with diameter of 6 mm (Bioanalyse Antimicrobial susceptibility test discs) were impregnated with 15 μL of sample solutions (0.5 mg/mL in DMSO) and placed on Muller Hilton agar plates. The plates were incubated at 37 °C for 24 h. Inhibition zones on the medium were evaluated in mm. All tests were performed in duplicate. DMSO (blank) was used as reference disc and the inhibition zones of tested materials were checked against with those reference discs. The susceptibility of the microorganisms was also tested with chloramphenicol disc (HIMEDIA Chloramphenicol C300 SD006-SCT) as a positive control.

2.3.4. Antimicrobial activity of film samples

Antimicrobial activity of films were investigated according to a previous study [31]. *Escherica coli* and *Staphylacoccus aureus* bacteria were studied. Firstly, bacterial suspensions of *Escherica coli* and *Staphylacoccus aureus* were obtained by culturing the bacteria in nutrient agar at 37 °C for 24 h. Then, bacterial suspensions were adjusted to a concentration of 10^8 CFU/mL against 0.5 Mc Farland and diluted to 10^5 CFU/mL with nutrient broth. 100 mg of each film samples were added to 5 mL of bacterial suspensions and shaken in Memmert water bath for 2 h at 37 °C. The bacterial suspensions with film samples were further diluted to 10^2 CFU/mL and 100 μL of them were inoculated onto Muller Hinton Agar and incubated at 37 °C for 24 h. A control sample, without the addition of film samples, was also tested using the same procedure. This procedure was performed separately for both types of bacteria.

2.4. Characterization of films

Tensile tests such as tensile modulus, elastic modulus and elongation at break were applied by using a Zwick Roell Z 020 tensile testing machine with 25 kN load cell model drawing cell and the pulling with a speed of 1 mm/min. The all tests were carried out at room temperature.

Water contact angle measurements were conducted using a Nano-Linker Contact Angle Measurement System (CA-500A) in combination with a Hamilton microsyringe equipped with a 22-gauge needle, employing the sessile drop mode. A 5 μL water droplet was carefully applied to the glass surface, and the image of the water droplet was captured 10 s after it contacted the sample surface. Differential scanning calorimetry (DSC) analyses were performed on PerkinElmer Diamond DSC from 30 °C to 200 °C with a heating rate of 10 ∘C min^{-1} under nitrogen flow.

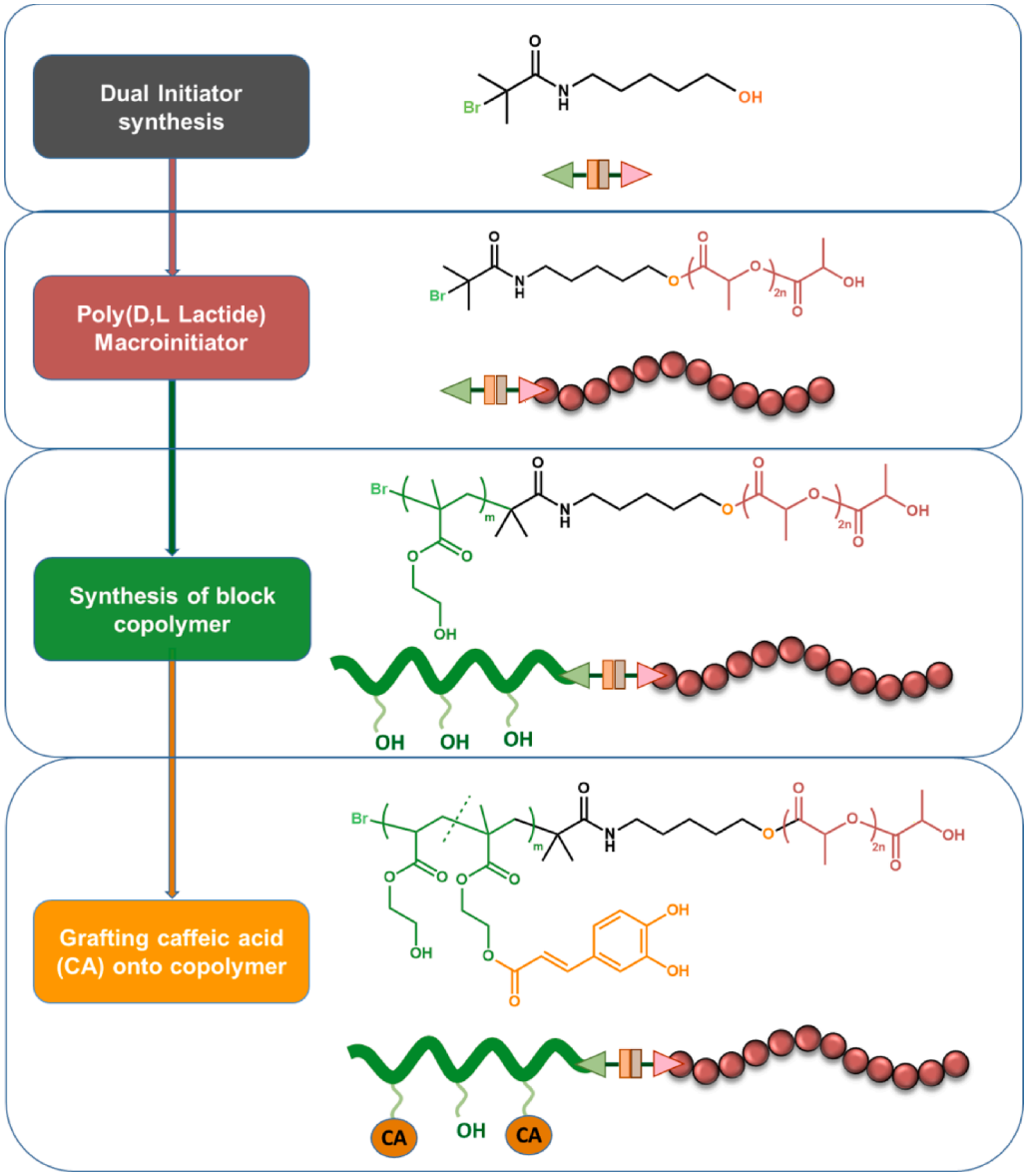

Fig. 1. Synthetic route for fabrication of PLA-*b*-PHEMA-*g*-CA.

3. Results and discussions

3.1. Synthesis of PLA-g-PHEMA-g-CA

Synthetic route for the fabrication of caffeic acid grafted block copolymer includes four steps as illustrated in Fig. 1. In the first step, dual initiator was synthesized to ensure the ring opening polymerization of D,L-lactide first and then atom transfer radical polymerization (ATRP) of HEMA. Secondly, PLA-Br was prepared from ROP of the monomer lactide initiated by hydroxyl group of the dual initiator and then used as macroinitiator in block copolymerization with HEMA monomer via ATRP in the third step. Finally, esterification reaction was carried out between the hydroxyl groups of PHEMA in block copolymer and carboxylic acid groups of caffeic acid.

In the first step, 2-bromo-*N*-(5-hydroxypentyl)2-methylpropanamide) (BNMP) was chosen as bifunctional initiator and successfully synthesized via amidation reaction between 5-amino-1-pentanol and 2-bromoisobutyryl bromide in the presence of triethyl amine. The structure of dual initiator was confirmed by using ^{1}H NMR spectroscopy (Figure S1). The characteristic peaks of the both precursors, 2-bromoisobutyryl bromide and 5-amino-1-pentanol, were observed at 1.96 ppm, and 3.29 and 3.66 ppm, respectively.

ROP of the lactide was carried out in a bulk polymerization initiated by the dual initiator (BNMP) in the presence of $Sn(Oct)_2$. Poly(D,L-lactide) macroinitiator bearing alkyl bromide end group (PLA-Br) was obtained with high yield (93 %). Subsequently, ATRP of HEMA resulted in PLA-*b*-PHEMA. Finally, caffeic acid was grafted to hydroxyl group of HEMA residues through a typical esterification reaction. Structure of all polymers were confirmed by FT-IR and NMR spectroscopies. As can be seen in Fig. 2, all FT-IR spectra of the all polymers showed 2945 cm^{-1} and 2994 cm^{-1} bands due to C–H stretching. PLA, PLA-*b*-PHEMA and PLA-*b*-PHEMA-*g*-CA displayed a distinctive vibration band at 1750 cm^{-1}due to the ester carbonyl of PLA. Both spectra of PLA-*b*-PHEMA and PLA-*b*-PHEMA-*g*-CA showed shoulder peaks at 1720 cm^{-1} due to the ester group of HEMA segment. Unlike the case of PLA and PLA-*b*-PHEMA, only PLA-*b*-PHEMA-*g*-CA displayed C = C stretching bands at 1593 cm^{-1}, 1633 cm^{-1} and 1637 cm^{-1} which were attributed to the aromatic and olefin groups of caffeic acid. The band at 3507 cm^{-1} in the spectra of PLA-*b*-PHEMA and PLA-*b*-PHEMA-*g*-CA was ascribed to the –OH groups of HEMA and caffeic acid. Due to C–H bending of PLA, a band at 1452 cm^{-1} was observed in all products. The bands at 1452 cm^{-1} 1185 cm^{-1} and 1082 cm^{-1} were ascribed to C–H bending, C-O bending and C-O-C alkoxy group of PLA respectively.

The chemical structures of the polymers were also confirmed by ^{1}H NMR spectroscopy (Fig. 3, S2 and S3). The number average molecular mass (M_n) of polymers was determined by end group analysis. Thus, M_n of PLA-Br was calculated by integration of methine proton signal (Figure S2, proton 1) from the repeating unit versus ω-bromo ester dimethyl proton signal (Figure S2, proton d) from end group. By using the ratio of polymer to end group integration, repeating unit of PLA-Br (DP = 88; 2n = 175) was calculated. Similarly, the repeating unit of PHEMA (m = 37) in the block copolymer was calculated by determining the ratio of the methine (–CH) proton (Figure S3, proton d) of PLA-Br repeating unit to $–CH_2$ proton (Figure S3, proton 4 or 5) of PHEMA. Molecular masses of the polymers were determined theoretically and by GPC; the results were given in Table 3. Since PLA-Br was soluble in THF and the block copolymers in DMF, two different GPC systems were used. Although there were some differences, the theoretical molecular masses were comparable with those determined from NMR analysis. The differences between theoretical molecular masses and NMR results were probably due to the errors from integration of very small peaks such as proton d in Figure S2. When the molecular masses obtained from GPC were compared to the others, the differences were acceptable for PLA-Br and PLA-*b*-PHEMA. However, molecular mass of PLA-*b*-PHEMA-*g*-CA was determined as 21.26 kDa and 89.00 kDa by NMR and GPC, respectively. This “pseudo” high molecular mass in GPC can be resulted from aggregation of the polymer chains due to interchain hydrogen bonding as reported MacDiarmid and coworkers [32]. Caffeic acid moiety of PLA-*b*-PHEMA-*g*-CA may be involved in interchain hydrogen bonding and caused similar “pseudo” high molecular mass in GPC. In addition to ‘pseudo’ high molecular mass in GPC, the difference between hydrodynamic volumes of polystyrene (PS) standards used for calibration and PLA-*b*-PHEMA-*g*-CA may result in higher molecular masses than expected ones. In a previous study, molecular mass differences between GPC results and theoretical values for some biopolymers, such as PLA and PCL were reported. In such cases, correction coefficient (ranged from 0.40 to 0.87) may be used to evaluate absolute molecular masses [33].

The UV–Vis absorption spectra of PLA-*b*-PHMEA and PLA-*b*-PHEMA-*g*-CA are shown in Fig. 4A. The spectrum of PLA-*b*-PHEMA-*g*-CA demonstrates characteristic absorption band between 375 nm – 275 nm due to grafted caffeic acid. PLA-*b*-PHMEA showed no absorption around this region due to the lack of chromophore group. This result supports conjugation of caffeic acid to the block copolymer.

A linear calibration curve (Fig. 4B) was generated by using a set of standard solutions with known concentrations of caffeic acid, in order to calculate the grafting degree of caffeic acid onto the polymer chain (r^2 = 0.993). The results of the calculation showed that the grafting degree was found to be 60.7 %.

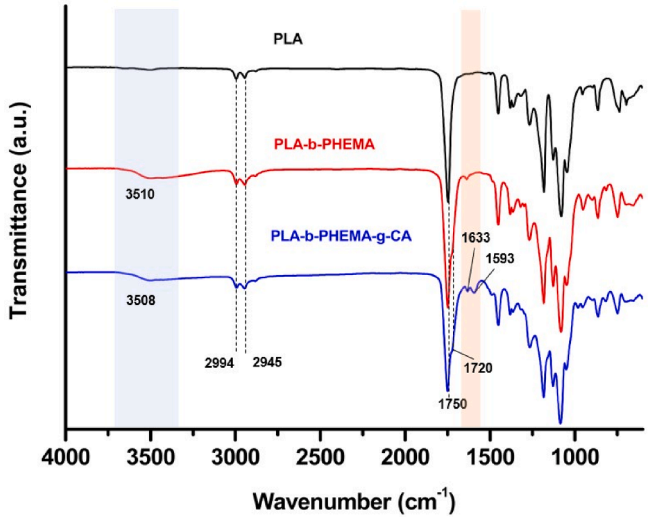

Fig. 2. Overlay of FTIR spectra of the polymers.

3.2. Antioxidant capacity and antimicrobial activity of polymers

The DPPH radical scavenging activities, calculated according to Equation (1), were plotted against the sample concentrations at eight different levels for antioxidant capacity determination. By using the linear regression line (y = ax + b) antioxidant activity of PLA-*b*-PHEMA-*g*-CA polymer was calculated by substituting the value of y with value of 50 in the regression equation as given Fig. 5. Result was defined as EC_{50} value (concentration that eliminates of 50 % DPPH• radical). While PLA-*b*-PHEMA showed no antioxidant activity, the PLA-*b*-PHEMA-*g*-CA polymer exhibited significant antioxidant activity with a DPPH radical scavenging capacity of 10.9 μg/mL (EC_{50}).

The results of our study showed that the DPPH radical scavenging activity of PLA-*b*-PHEMA-*g*-CA was comparable to that of well-known positive controls such as ascorbic acid and butylated hydroxyanisol, which have reported activities of 1.09 μg/mL and 3.74 μg/mL, respectively, in existing literature [34].

In a previous study reported from Damasceno et al., one of the antioxidant mechanisms of the caffeic acid molecule is through the reduction of hydroxyls attached to the benzene ring by radicals. The

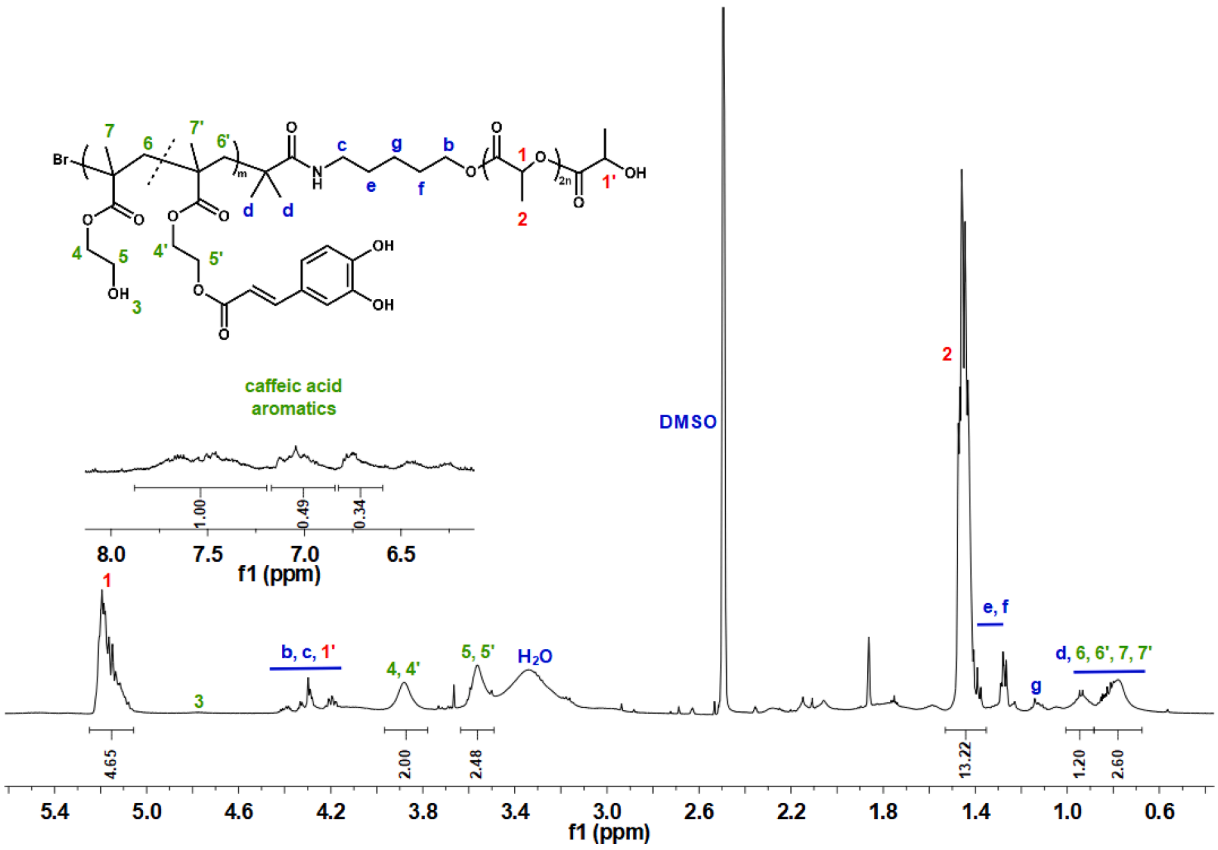

Fig. 3. ^{1}H NMR spectrum of PLA-*b*-PHEMA-*g*-CA (DMSO-d_6).

Table 3
Molecular masses of the polymers.

Polymer	$M_{n,theo}$ (kDa)	$M_{n,NMR}$ (kDa)	$M_{n,GPC}$ (kDa)	PDI	GPC Eluent
PLA-Br	9.65	12.85	18.19	1.80	THF
PLA-*b*-PHEMA	13.44	17.70	18.77	1.53	DMF
PLA-*b*-PHEMA-*g*-CA	16.27	21.26	89.00	1.38	DMF

resulting radical becomes stable by ring resonance [35]. In this study, caffeic acid was linked to the polymer chain via the carboxylic acid group, and unmodified hydroxyl groups participate in this antioxidant mechanism.

The antimicrobial activities of the polymers were evaluated using *E. coli* and *S. aureus* bacteria. Inhibition zones of disks containing the polymers or the antibiotic were measured by Scan 1200 HD automatic colony counter and expressed as average inhibition zone in mm. The inhibition zones of polymers were given in Table 4. While PLA-*b*-PHEMA showed no inhibition zone, PLA-*b*-PHEMA-*g*-CA demonstrated

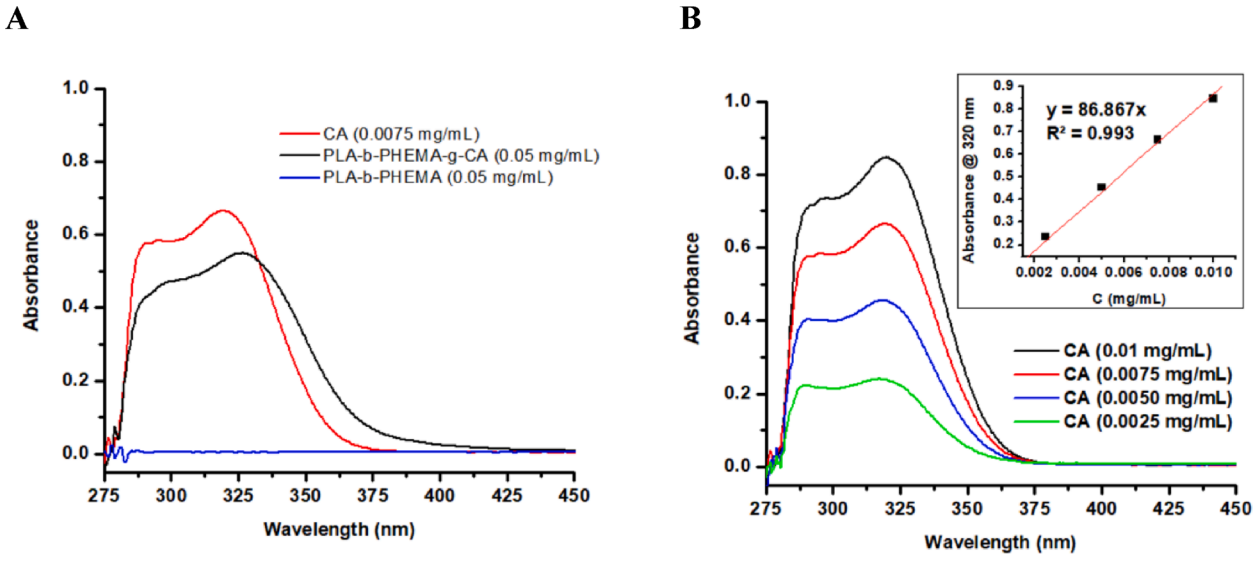

Fig. 4. (A) UV–Vis spectra of CA, PLA-*b*-PHMEA and PLA-*b*-PHEMA-*g*-CA; (B) UV–Vis spectra and calibration curve (at 320 nm) of caffeic acid standards.

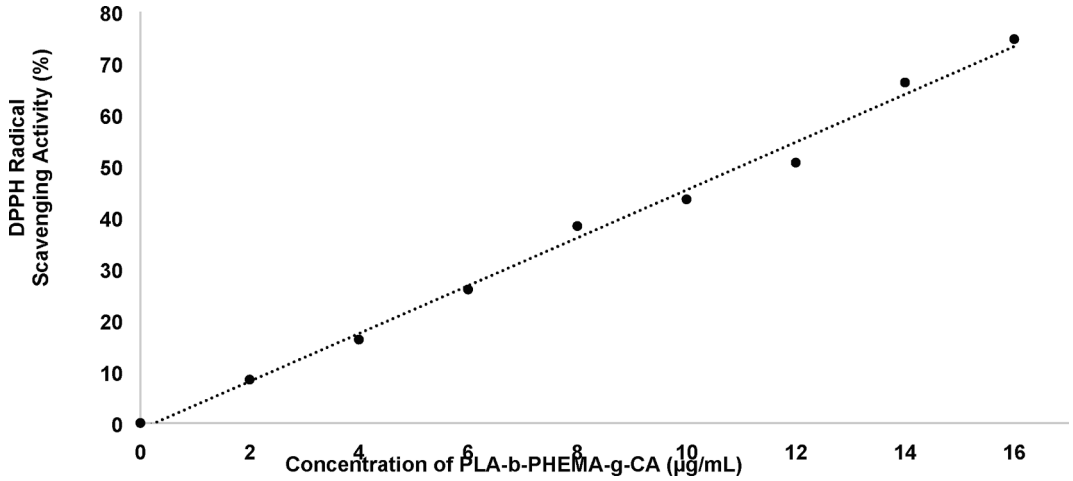

Fig. 5. Linear regression curve of PLA-*b*-PHEMA-*g*-CA.

antimicrobial activity with a small inhibition zone against *S. aureus* and *E. coli* when compared to positive control (chloramphenicol). This may be due to the low water solubility and, in turn, the diffusion difficulties of PLA-*b*-PHEMA-*g*-CA. The PLA-*b*-PHEMA-*g*-CA was designed as non-immigratory agent; thus, the small inhibition zone was not surprising. On the other hand, as can be seen in Figure S5, inhibition zone of PLA-*b*-PHEMA-*g*-CA demonstrate that material has antimicrobial activity against *E. coli* and *S. aureus*.

3.3. Characterization of films prepared from blends of PLA/PLA-b-PHEMA-g-CA

Films with different compositions were obtained by blending regular PLA and caffeic acid containing PLA copolymers (Table 5). The water contact angle measurements were employed to evaluate the surface hydrophilicity of PLA films, as shown in Fig. 6. The neat PLA film and the film with 3 % PLA-*b*-PHEMA-*g*-CA had similar contact angles around 60.95° and 62.77 °, respectively. Further increase in content of PLA-*b*-PHEMA-*g*-CA resulted in decrease in water contact angles (Table 5), since PLA-*b*-PHEMA-*g*-CA is relatively hydrophilic compared to neat PLA.

Young's modulus, tensile strength and elongation at break of the film samples were determined by stress–strain test and summarized in Table 6. The results indicate that addition of PLA-*b*-PHMEA-*g*-CA decreased both the modulus and elongation at break in comparison to PLA-0 % PHC film. The decrease Young's moduli can be concluded that the block copolymer has plasticizer effect and the material becomes more flexible. This is beneficiary for PLA films since commercial PLA is too rigid for packaging materials because of high crystallinity and requires plasticizer. This result is consistent with literature examples [36,37]. However, surprisingly the tensile strength and elongation at break were lower than those of neat PLA. Relatively higher hydrophilic nature of the block copolymer may cause phase separation and, in turn, decrease in the mechanical properties. The main purpose of the study is to fabricate a PLA material with antioxidant and antibacterial properties instead of improving mechanical properties.

Table 4
Antimicrobial activity of block copolymers against *E. coli* and *S. Aureus*.

	Average Inhibition Zones (mm)	
Samples	***Escherichia coli***	***Staphylococcus aureus***
PLA-*b*-PHEMA	8.1	–
PLA-*b*-PHEMA-*g*-CA	8.8	9.1
Chloramphenicol	26.2	24
Blank (DMSO)	8.0	–

Table 5
Water contact angle measurements of the PLA films.

Name	PLA %	PEG %	PLA-*b*-PHEMA-*g*-CA (PHC)	Water contact angle (°)
PLA-0 % PHC	95	5	0	61.0
PLA-3 % PHC	92	5	3	62.8
PLA-5 % PHC	90	5	5	56.3
PLA-7 % PHC	88	5	7	54.2
PLA-10 % PHC	85	5	10	41.2

To gain further insight, thermal properties of the films were evaluated with DSC measurements. As no T_m was observed in its thermogram, PLA-*b*-PHEMA-*g*-CA is considered as non-crystalline. On the other hand, the neat PLA and the films exhibited melting temperature ranging between 167–177 °C. There is a slightly decline trend in the T_m values with increasing block copolymer content as can be seen in Fig. 7. This is because the presence of the block copolymer affects arrangement of PLA polymer chains and the packing density of PLA yielding slight decrease in T_m. Similar behavior was observed by others when PLA was grafted with maleic acid [38].

3.4. Bioactivity of films

The antioxidant properties of the film samples were investigated by assessing its ability to scavenge the stable DPPH radical. While the PLA-*b*-PHEMA block copolymer initially lacked inherent antioxidant properties, grafting of caffeic acid enhance the antioxidant capability of block copolymer. The PLA-*b*-PHEMA-*g*-CA polymer was blended with commercial PLA to impart antioxidant properties to the film samples, and it was found that the antioxidant activity increased with the increasing ratio of this bioactive polymer in films, according to the results as given in Table 7. The hydroxyl groups attached to the benzene ring of caffeic acid were identified as having the capacity to reduce the stable DPPH radical [39]. Caffeic acid donates electrons or hydrogen to free radicals, converting them into more stable forms. The resulting form of caffeic acid is also stable, thanks to the resonance effect of the aromatic ring. In this study, caffeic acid was covalently grafted from the carboxyl group to the polymer, and the available hydroxyl groups in the molecule interacted with radicals, demonstrating antioxidant properties without diffusion into the environment.

The incorporation of grafted copolymer in PLA films (PLA-*b*-PHEMA-

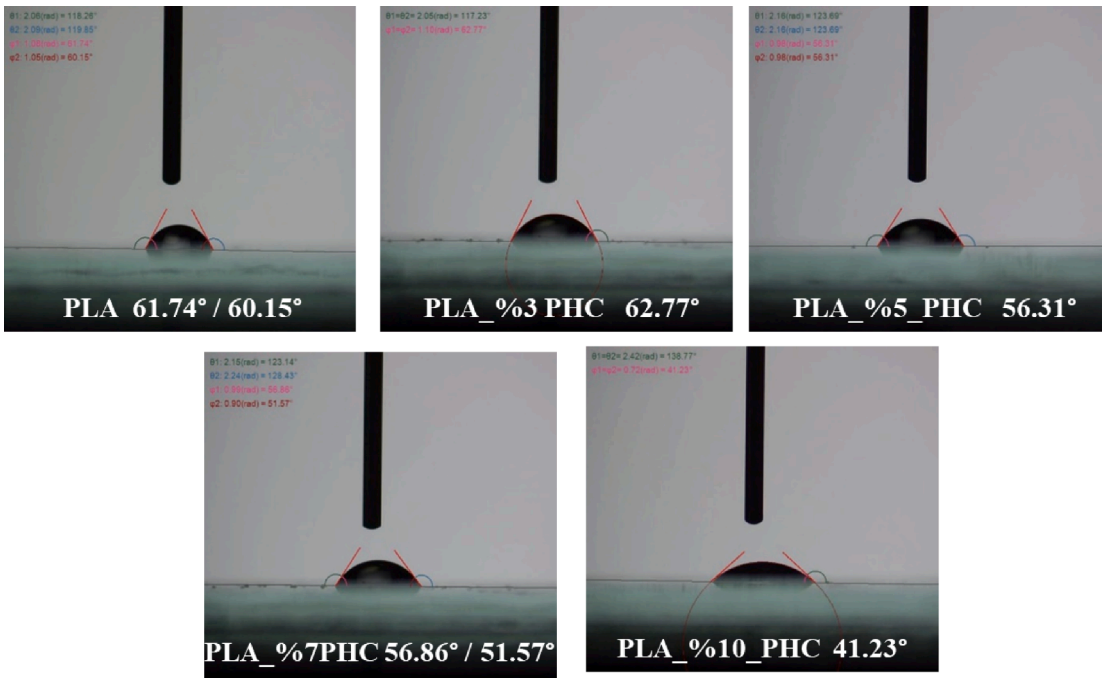

Fig. 6. Water contact angle measurements of PLA films blended with various amount of PLA-*b*-PHEMA-*g*-CA.

Table 6
Tensile test results of films.

Sample	Young's modulus (MPa)	Tensile strength (MPa)	Elongation @ break (%)
PLA-0 % PHC	1900	30.1	5.29
PLA-3 % PHC	1007	29.42	3.96
PLA-5 % PHC	720	14.71	1.79
PLA-7 % PHC	661	15.89	2.76
PLA-10 % PHC	606	7.32	1.1

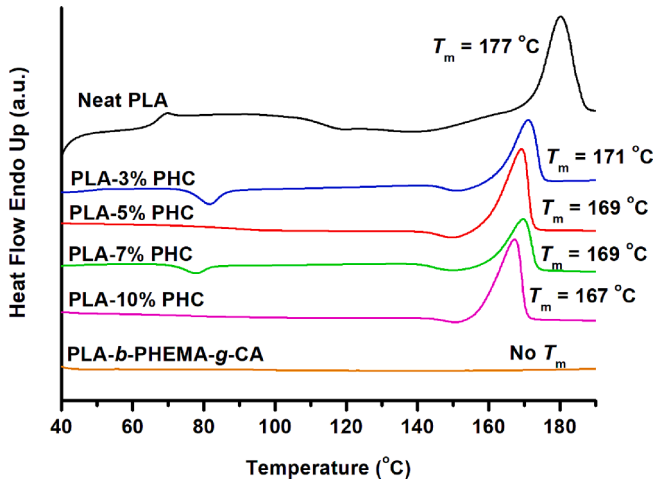

Fig. 7. Second heating DSC thermograms of film samples and precursor polymers.

Table 7
Antioxidant activity of film samples.

Sample	Scavenging activity on DPPH• (%)
Blank	0,737 ± 0,004
PLA-3 % PHC	6,248 ± 0,008
PLA-5 % PHC	50,851 ± 0,752
PLA-7 % PHC	55,55 ± 0,812
PLA-10 % PHC	84,989 ± 0,432

g-CA) has been shown to decrease *S. aureus* bacterial growth with increasing concentration as shown in Figure S6. The number of colonies of *S. aureus* were $69x10^2$, $28x10^2$, $13x10^2$, $5x10^2$, $3x10^2$ for blank, 3 %, 5 %, 7 % and 10 % PHC, respectively. The plates were too many colonies to count (TNCC) the cultures of *E. coli*, however, it appeared that the density of colonies on the plates had slightly decreased. These results suggest that the films exhibited stronger antimicrobial activity against *S. aureus* than *E. coli*, likely due to the differences in cell wall structure between gram-positive and gram-negative bacteria.

4. Conclusions

The present study describes the synthesis and characterization of a caffeic acid grafted poly(lactide)-*block*-poly(2-hydroxyethyl methacrylate) copolymer (PLA-*b*-PHEMA). Additionally, the study investigates the mechanical and bioactive properties of films produced by blending various amounts of the grafted copolymer with commercial PLA. The study began with the successful synthesis of a dual initiator, BNMP, that contains both a hydroxyl group for ring-opening polymerization (ROP) and an alkyl bromide for atom transfer radical polymerization (ATRP). ROP of lactide was achieved exclusively through chain growth initiated from the hydroxyl group of the initiator. Then, 2-hydroxyethyl methacrylate (HEMA) was polymerized by controlled radical polymerization. Eventually, caffeic acid was grafted onto PHEMA segments of the copolymer via Steglich esterification reaction. The synthesized compounds were characterized using NMR, IR, and UV–Vis spectroscopies, and their bioactivities, including anti-oxidant capacity and antibacterial effect, were investigated. The caffeic acid grafted copolymer (PLA-*b*-PHEMA-*g*-CA) exhibited antimicrobial activity against *E. coli* and *S.*

aureus in in-vitro tests. The approach developed in the current work can be extended by utilizing various polymer backbones and bioactive pendant groups. The resulting polymer can then be blended with other types of polymers to create new materials with new properties for not only packaging and but also many other applications.

CRediT authorship contribution statement

Gamze Düz: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sinem Sipahioğlu Kara:** Writing – review & editing, Formal analysis. **Özlem Yılmaz:** Writing – review & editing, Formal analysis, Conceptualization. **Muhammet U. Kahveci:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

This paper is dedicated to the memory of our dear professor Yusuf Yagci who passed away on January 30, 2023. This work was financially supported by Istanbul Technical University Scientific Research Projects (ITU BAP, Project # TYL-2021-43077).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eurpolymj.2024.113056.

References

[1] L.K. Ncube, A.U. Ude, E.N. Ogunmuyiwa, R. Zulkifli, I.N. Beas, An overview of plastic waste generation and management in food packaging industries, Recycling 6 (1) (2021) 12.
[2] T. Narancic, K.E. O'Connor, Plastic waste as a global challenge: are biodegradable plastics the answer to the plastic waste problem? Microbiology 165 (2) (2019) 129–137.
[3] A.C. Mendes, G.A. Pedersen, Perspectives on sustainable food packaging:- is bio-based plastics a solution? Trends Food Sci. Technol. 112 (2021) 839–846.
[4] S. Alavi, S. Thomas, K. Sandeep, N. Kalarikkal, J. Varghese, S. Yaragalla, Polymers for packaging applications, CRC Press, 2014.
[5] G. Li, M. Zhao, F. Xu, B. Yang, X. Li, X. Meng, L. Teng, F. Sun, Y. Li, Synthesis and biological application of polylactic acid, Molecules 25 (21) (2020) 5023.
[6] L.T. Sin, B.S. Tueen, Polylactic acid: a practical guide for the processing, manufacturing, and applications of PLA, William Andrew 2019.
[7] L. Shao, Y. Xi, Y. Weng, Recent advances in PLA-based antibacterial food packaging and its applications, Molecules 27 (18) (2022) 5953.
[8] R. Ordoñez, L. Atarés, A. Chiralt, Biodegradable active materials containing phenolic acids for food packaging applications, Compr. Rev. Food Sci. Food Saf. 21 (5) (2022) 3910–3930.
[9] S. Roy Goswami, S. Sudhakaran Nair, S. Wang, N. Yan, Recent progress on starch maleate/polylactic acid blends for compostable food packaging applications, ACS Sustain. Chem. Eng. 10 (1) (2022) 3–15.
[10] L.K. Ncube, A.U. Ude, E.N. Ogunmuyiwa, R. Zulkifli, I.N. Beas, Environmental impact of food packaging materials: A review of contemporary development from conventional plastics to polylactic acid based materials, Materials 13 (21) (2020) 4994.
[11] L.F. Mortensen, I. Tange, Å. Stenmarck, A. Fråne, T. Nielsen, N. Boberg, F. Bauer, Plastics, the circular economy and Europe′ s environment-A priority for action, (2021).
[12] P. Europe, Plastics–the facts 2020, PlasticEurope 1 (2020) 1–64.
[13] S. Yildirim, B. Röcker, M.K. Pettersen, J. Nilsen-Nygaard, Z. Ayhan, R. Rutkaite, T. Radusin, P. Suminska, B. Marcos, V. Coma, Active Packaging Applications for Food, Compr. Rev. Food Sci. Food Saf. 17 (1) (2018) 165–199.
[14] R. Ordoñez, L. Atarés, A. Chiralt, Multilayer antimicrobial films based on starch and PLA with superficially incorporated ferulic or cinnamic acids for active food packaging purposes, Food Chemistry Advances 2 (2023) 100250.
[15] E. Moll, C. Gonzalez-Martinez, A. Chiralt, Release and antibacterial action of phenolic acids incorporated into PHBV films, Food Packag. Shelf Life 38 (2023).
[16] D. Rigotti, M. Soccio, A. Dorigato, M. Gazzano, V. Siracusa, G. Fredi, N. Lotti, Novel biobased polylactic acid/poly(pentamethylene 2,5-furanoate) blends for sustainable food packaging, ACS Sustain. Chem. Eng. 9 (41) (2021) 13742–13750.
[17] G. Lopez-Carballo, J. Gomez-Estaca, R. Catala, P. Hernandez-Munoz, R. Gavara, Active antimicrobial food and beverage packaging, in: K.L. Yam, D.S. Lee (Eds.), Emerging Food Packaging Technologies: Principles and Practice2012, pp. 27-54.
[18] R. Gavara, Practical guide to antimicrobial active packaging, Smithers Pira (2015).
[19] K. Sadeghi, J. Seo, Photografting of biochelator onto polypropylene film as an antioxidant clean label, Food Chem. 351 (2021).
[20] C.B. Contreras, G. Charles, R. Toselli, M.C. Strumia, Antimicrobial active packaging, CRC Press, Biopackaging, 2017, pp. 36–58.
[21] A. Aljawish, L. Muniglia, A. Klouj, J. Jasniewski, J. Scher, S. Desobry, Characterization of films based on enzymatically modified chitosan derivatives with phenol compounds, Food Hydrocoll. 60 (2016) 551–558.
[22] V. Mourya, N.N. Inamdar, Chitosan-modifications and applications: Opportunities galore, React. Funct. Polym. 68 (6) (2008) 1013–1051.
[23] D. Arrua, M.C. Strumia, M.A. Nazareno, Immobilization of caffeic acid on a polypropylene film: synthesis and antioxidant properties, J. Agric. Food Chem. 58 (16) (2010) 9228–9234.
[24] L.J. Bastarrachea, D.E. Wong, M.J. Roman, Z. Lin, J.M. Goddard, Active packaging coatings, Coatings 5 (4) (2015) 771–791.
[25] F. Chen, Z. Shi, K. Neoh, E. Kang, Antioxidant and antibacterial activities of eugenol and carvacrol-grafted chitosan nanoparticles, Biotechnol. Bioeng. 104 (1) (2009) 30–39.
[26] B.R. Albuquerque, S.A. Heleno, M.B.P. Oliveira, L. Barros, I.C. Ferreira, Phenolic compounds: Current industrial applications, limitations and future challenges, Food Funct. 12 (1) (2021) 14–29.
[27] F.F. Wolf, N. Friedemann, H. Frey, Poly (lactide)-block-poly (HEMA) block copolymers: an orthogonal one-pot combination of ROP and ATRP, using a bifunctional initiator, Macromolecules 42 (15) (2009) 5622–5628.
[28] S.E. Averick, S.K. Dey, D. Grahacharya, K. Matyjaszewski, S.R. Das, Solid-phase incorporation of an ATRP initiator for polymer–DNA biohybrids, Angew. Chem. Int. Ed. 53 (10) (2014) 2739–2744.
[29] Ingeo™ Biopolymer 2500HP Technical Data Sheet, NatureWorks, 15305 Minnetonka Blvd., Minnetonka, MN 55345.
[30] H. Kim, P.K. Panda, K. Sadeghi, J. Seo, Poly (vinyl alcohol)/hydrothermally treated tannic acid composite films as sustainable antioxidant and barrier packaging materials, Prog. Org. Coat. 174 (2023) 107305.
[31] L. Zhang, C. Huang, Y. Xu, H. Huang, H. Zhao, J. Wang, S. Wang, Synthesis and characterization of antibacterial polylactic acid film incorporated with cinnamaldehyde inclusions for fruit packaging, Int. J. Biol. Macromol. 164 (2020) 4547–4555.
[32] W. Zheng, M. Angelopoulos, A.J. Epstein, A.G. MacDiarmid, Experimental evidence for hydrogen bonding in polyaniline: mechanism of aggregate formation and dependency on Oxidation State, Macromolecules 30 (10) (1997) 2953–2955.
[33] B. Kost, M. Socka, K. Cichoń, M. Brzeziński, M. Basko, T. Biela, Molar mass determination via linear regression from SEC measurements: The case of polylactide, polycaprolactone and poly (2, 2-dimethylthrimethylene carbonate), Polym. Adv. Technol. 35 (1) (2024) e6296.
[34] N. Belkacem, R. Djaziri, F. Lahfa, I. El-Haci, Z. Boucherit, Phytochemical screening and in vitro antioxidant activity of various Punica granatum l, Peel Extracts from Algeria: A Comparative Study, Phytothérapie 6 (12) (2014) 372–379.
[35] S.S. Damasceno, N.A. Santos, I.M. Santos, A.L. Souza, A.G. Souza, N. Queiroz, Caffeic and ferulic acids: An investigation of the effect of antioxidants on the stability of soybean biodiesel during storage, Fuel 107 (2013) 641–646.
[36] Y. Eom, B. Choi, S.-I. Park, A study on mechanical and thermal properties of PLA/PEO blends, J. Polym. Environ. 27 (2) (2019) 256–262.
[37] S. Sharma, A.K. Jaiswal, B. Duffy, S. Jaiswal, Ferulic acid incorporated active films based on poly (lactide)/poly (butylene adipate-co-terephthalate) blend for food packaging, Food Packag. Shelf Life 24 (2020) 100491.
[38] H. Jang, S. Kwon, S.J. Kim, S.-I. Park, Maleic Anhydride-Grafted PLA Preparation and Characteristics of Compatibilized PLA/PBSeT Blend Films, Int. J. Mol. Sci. 23 (13) (2022) 7166.
[39] K.M.M. Espíndola, R.G. Ferreira, L.E.M. Narvaez, A.C.R. Silva Rosario, A.H.M. da Silva, A.G.B. Silva, A.P.O. Vieira, M.C. Monteiro, Chemical and Pharmacological Aspects of Caffeic Acid and Its Activity in Hepatocarcinoma, Frontiers in Oncology 9 (2019).