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for

Hyperbranched Polyesters as Biodegradable and Antibacterial Additives

Hadi Bakhshi*, Seema Agarwal*

Macromolecular Chemistry II and Bayreuth Center for Colloids and Interfaces, University of Bayreuth, Universitätsstraße 30, 95440, Bayreuth, Germany

*Corresponding author: E-mail: (H. Bakhshi) hadi.bakhshi@uni-bayreuth.de, (S. Agarwal) agarwal@uni-bayreuth.de, Fax: +49-921-553393; Tel: +49-921-553397.

1. Experimental

1.1. Materials

1,6-hexandiol diacrylate (HDA, 99%) and piperazine (PZ, 99%) was supplied by ABCR. 1-(2-Aminoethyl)piperazine (AEPZ, 99%) and diethylamine (DEA, 99%) were bought from Acros Chemicals. Poly(caprolactone) (PCL, Capa™ 6800, T_m: 58-60°C) was obtained from Perstorp. Sterase EL-01 from *Thermomyces languinosus* was received from ASA Spezialenzyme.

1.2. Synthesis of hyperbranched poly(amino-ester) (HPAE)

HPAE was synthesized using HDA (A_2) and AEPZ (B_3) as di- and tri-functional monomers, respectively. For this purpose, a three-neck reaction flask equipped with a dropping funnel, an Ar inlet, a condenser and an oil bath was charged with HDA (22.86 g, 100 mmol, 1 Eq.) and THF (100 mL), and bubbled with Ar for 10 min to remove the dissolved O_2. A solution of AEPZ (8.70 g, 67 mmol, 1 Eq.) in THF (100 mL) bubbled with Ar for 10 min was slowly added to the flask through the dropping funnel. The reaction solution was warmed up to 50-55°C and stirred for 4 days. Then, the flask was charged with DEA (0.19 g, 5 mmol) dissolved in THF (5 mL) and stirred for another day to end-cap the polymer chains. Finally, THF and unreacted DEA were removed via a vacuum rotary evaporator at 50°C and the product was completely dried at 40°C in a vacuumed oven overnight. The obtained HPAE (31.24` g, yield of 100%) was a colorless viscose liquid. FTIR (1/λ, cm^{-1}): 3314 (N–H, ν), 2938 and 2814 (C–H, ν), 1729 (C=O, ν), 1458 and 1300 (C–H, δ), 1253 (C–N, ν), 1173 and 1010 (C–O, ν).

1.3. Synthesis of linear poly(amino-ester) (LPAE)

LPAE was prepared through same procedure mentioned for HPAE except using di-functional PZ (B_2, 8.70 g, 100 mmol, 1 Eq.) instead of AEPZ. The obtained LPAE was a yellow solid (31.24 g, yield of 100%). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.37 (tt, 4H, $OCH_2CH_2\mathit{CH_2}$), 1.63 (tt, 4H, $OCH_2\mathit{CH_2}$), 2.49 (m, 12H, $\mathit{CH_2}CO$ and $NCH_2\mathit{CH_2}N$), 2.68 (t, 4H, $\mathit{CH_2}CH_2CO$), 4.07 (t, 4H, $O\mathit{CH_2}$). ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm): 22.6 ($OCH_2CH_2\mathit{CH_2}$), 28.5 ($OCH_2\mathit{CH_2}$), 32.3 ($\mathit{CH_2}CO$), 52.8 ($NCH_2\mathit{CH_2}N$), 53.5 ($\mathit{CH_2}CH_2CO$), 64.4 ($O\mathit{CH_2}$), 172.5 (CO). FTIR (1/λ, cm^{-1}): 2938 and 2816 (C–H, ν), 1724 (C=O, ν), 1458 and 1312 (C–H, δ), 1254 (C–N, ν), 1177 and 1011 (C–O, ν).

1.4. Quaternization of HPAE and LPAE

QAS-functionalized PAEs were prepared via alkylation reaction of the tertiary amines within their backbone with alkyl bromides [1]. As a general procedure, a solution of 5 g of PAE and alkyl bromide (3 Eq. regarding 1 Eq. tertiary amines) in THF (50 mL) was stirred at room temperature for one week. THF was then removed by a vacuum rotary evaporator at 50°C and the resulting mass was washed three times with *n*-hexane to remove the excess alkyl bromide, and dried at 50°C in a vacuumed oven overnight.

HPAE-C4Br: The product was a yellow gum (6.31 g, yield of 67%) with high tackiness. The yield of quaternization was 33% according to ^{1}H-NMR spectroscopy. FTIR (1/λ, cm^{-1}): 2936, 2859 and 2813 (C–H, ν), 2690 and 2600 (N^{+}C–H, ν), 1729 (C=O, ν), 1458, 1355 and 1300 (C–H, δ), 1250 (C–N, ν), 1181, 1152 and 1010 (C–O, ν).

HPAE-C8Br: The product was a yellow gum (5.55 g, yield of 50%) with high tackiness. The yield of quaternization was 33% according to ^{1}H-NMR spectroscopy. FTIR (1/λ, cm^{-1}): 2928, 2855 and 2812 (C–H, ν), 2690 and 2600 (N^{+}C–H, ν), 1728 (C=O, ν), 1458 and 1301 (C–H, δ), 1250 (C–N, ν), 1181 and 1011 (C–O, ν).

HPAE-C12Br: The product was a yellow gum (4.73 g, yield of 36%) with high tackiness. The yield of quaternization was 21% according to ^{1}H-NMR spectroscopy. FTIR (1/λ, cm^{-1}): 2925, 2851 and 2813 (C–H, ν), 2690 and 2600 (N^{+}C–H, ν), 1728 (C=O, ν), 1458 and 1300 (C–H, δ), 1251 (C–N, ν), 1160 and 1011 (C–O, ν).

LPAE-C8Br: The product was a light brown solid (4.18 g, yield of 37%). The yield of quaternization was 4% according to ^{1}H-NMR spectroscopy. FTIR (1/λ, cm^{-1}): 2937 and 2820 (C–H, ν), 2692 and 2596 (N^{+}C–H, ν), 1724 (C=O, ν), 1461 and 1315 (C–H, δ), 1258 and 1234 (C–N, ν), 1177 and 1011 (C–O, ν).

1.5. Blending of PCL and Q-HPAE

PCL and HPAE-C8Br (90/10 and 80/20 wt/wt) were blended using a micro-compounder from Xplore (model MC 5, Netherlands) consisting two co-rotating conical screws under N_2 atmosphere with the rotation of 50 rpm at 125°C for 5-10 min. Free stand films of the blends with a thickness of 1 mm were obtained using a hot press operating at 125 °C.

1.6. Instruments

A Bruker spectrometer (model Avance 300, Germany) was used to record ^{1}H-NMR and ^{13}C-NMR spectra at room temperature using $CDCl_3$ or DMSO-d_6 as solvents. FTIR spectra were obtained using a Digilab spectrometer (model Excalibur FTS-3000 series, USA) equipped with an ATR unit. Molecular weight distribution of the samples was obtained by gel permeation chromatography (GPC) in DMF or $CHCl_3$ using a PSS-SDV pre-column (8 mm × 50 mm) and a PSS-SDV column (linear XL, 5 µm, 8 mm × 300 mm) with the rate of 0.5 mL/min at 40°C. The data was evaluated by Win GPC Unity software (build 6807) using polystyrene as the standards.

A Netzsch thermogravimetric analyzer (TGA, model TG 209 F1 Libra, Germany) operating at a heating rate of 10 °C/min under N_2 atmosphere was used to study the thermal degradation of the samples. Glass transition temperature (T_g) and melting point (T_m) of the samples were obtained from differential scanning calorimetry (DSC, Mettler Toledo, model DSC 821e, Switzerland) at a heating rate of 10°C/min under N_2 atmosphere.

The tensile properties of PCL blends was evaluated on a Zwick/Roell testing machine (model Z0.5) equipped with a load cell of 200 N and pneumatic grips, at 21°C and a relative humidity of 20%. The compression-molded films with a thickness of 1 mm were stamped-cut

into dog-bones shaped specimens having a width of 2 mm and a length of 20 mm. A digital micrometer was used to accurately measure the width and thickness of specimens. The crosshead speed was set at 50 mm/min. Data was evaluated by testXpert II software. The reported values are an average of at least four specimens for each sample.

Field emission scanning electron microscopy (FE-SEM) was done by a Zeiss instrument (model LEO 1530 Gemini, Germany) equipped with an energy-dispersive X-ray spectrometer (EDX, Thermo Scientific, model UltraDry, USA). Standard holders were used for mounting the samples using conductive adhesion graphite-pad (Plano) and vapor-coated with carbon using a Balzers Union coater (model MED 010).

A drop shape analyzer (Krüss, model DSA25E, Germany) was used to measure the contact angle of water droplets (2.0 µL) on the samples. The images were analyzed through Advance drop shape software (version 1.3.1.0) using Sessile drop orientation and Ellipse (Tangante^{-1}) fitting mode. An average of five measurements is reported.

1.7. Methods

The bulk hydrophilicity of PCL blends was evaluated via measuring the water absorption. For that end, completely dried and accurately weighted samples (80-120 mg) were immersed in 5 mL of Milli-Q at room temperature. After that, samples were removed, gently wiped by a tissue paper to remove surface water and immediately weighted. The water absorption values were calculated by the following equation:

$$Water\ absorption = \left(m_w - m_d\right)/m_d \quad (1)$$

where m_w and m_d are the weights of wet and dried samples, respectively.

The crystallinity (χ) of PCL blends was calculated according to the melting enthalpy (ΔH_m) values using the following equation:

$$\chi\ (\%) = \frac{\Delta H_{m\ (norm)}}{\Delta H_{m\ (crys)}} \times 100 \quad (2)$$

where $\Delta H_{m(norm)}$ is the normalized melting enthalpy obtained by dividing the ΔH_m value to the weight fraction of PCL in blends, and $\Delta H_{m(crys)}$ is the theoretical melting enthalpy of a 100% crystalline PCL taken as 136 J/g [2].

The antibacterial activity of the samples was studied via agar diffusion (Kirby-Bauer) and shaking flask assays. Gram-negative *Escherichia coli* (*E. coli*, DSM No. 1077, K12 strain 343/113, DSMZ) and Gram-positive *Bacillus subtilis* (*B. subtilis*, DSM No. 2109, ATCC 11774, ICI 2/4 strain, DSMZ) were used as bacteria. They were pre-cultured in nutrient broth in a shaking-oven at 37 °C overnight before assays. The nutrients were respectively CASO-Bouillon (30 g/L) and Meat Extract-Pepton (5 and 3 g/L). The OD of the bacteria suspensions (200 µL) were measured at 600 nm using a photometer (PerkinElmer, model Lambda XLS, Germany) to be able to dilute them to desired concentrations. Counting bacteria via serial dilution method on nutrient agar plate showed that an absorbance of 0.125 is equal to a concentration of 2×10^8 CFU/mL and 10^8 CFU/mL, respectively for *E. coli* and *B. subtilis*.

Agar diffusion (Kirby-Bauer) assay was used to determine the possible leaching of QAS-PAEs. For that purpose, paper discs containing 100 µg of each QAS-OAEs sample were prepared by pouring 10 µL of their solution in DMF (10 g/L) on blank discs (Oxoid, model CT0998B, UK, 6 mm diameter) and drying in a vacuum oven at 80°C overnight. A disc poured with pure DMF was also prepared as blank. PCL blend films were also cut to quadrangular form (5×5 mm). Samples were placed on nutrient agar plate seeded with 10^5 CFU of bacteria and incubated at 37°C overnight. Inhibition zone where no bacterial growth takes place was measured visually [3-7]. This test was run in triplicate. To check that if the bacteria in contact with the samples are killed or not, samples were carefully transferred to new nutrient agar plates, rubbed on the surface and incubated at 37°C overnight. Formation of the bacteria colonies on the rubbed area was an indication for survived bacteria in contact with samples.

Shaking flask method was employed to study the non-leaching bactericidal activity of the samples [6, 8, 9]. For this purpose, samples were put into a flask having 1 mL of PBS (pH=7.4) containing 10^5 CFU/mL of bacteria in separate vials and incubated with the rotation of 200 rpm at 37°C for 90 min. Then, the bacteria content of each vial was determined by serial dilution counting on nutrient agar plates. A blank flask (only bacteria) was run simultaneously. This test was run in triplicate.

For studying the contact antibacterial activity of PCL blends, 100 µL of bacteria suspensions with a concentration of 10^5 CFU/mL were transferred on samples (5×5 mm) and left in the biological hood for 3 hours to dry. Then, the surface of samples was completely covered with a thin layer of nutrient agar gel and incubated at 37°C overnight. After removing the agar gel layer, the surface of samples was investigated by SEM to check the possible growth of bacteria [10].

To study the degradability of PCL blends, accurately weighted sample (130-150 mg) were immersed in 5 mL of PBS (pH=7.4) containing NaN_3 (0.05 g/L) without or with Esterase (0.5 g/L) and placed in a shaking incubator with the rotation of 50 rpm at 37°C for 1-14 days. PBS medium was refreshed each day. After that, samples were removed, washed with water, lyophilized and weighed. The remaining solid was also characterized by GPC.

2. Results and Discussion

2.1. Studying the polyaddition reaction via FTIR spectroscopy

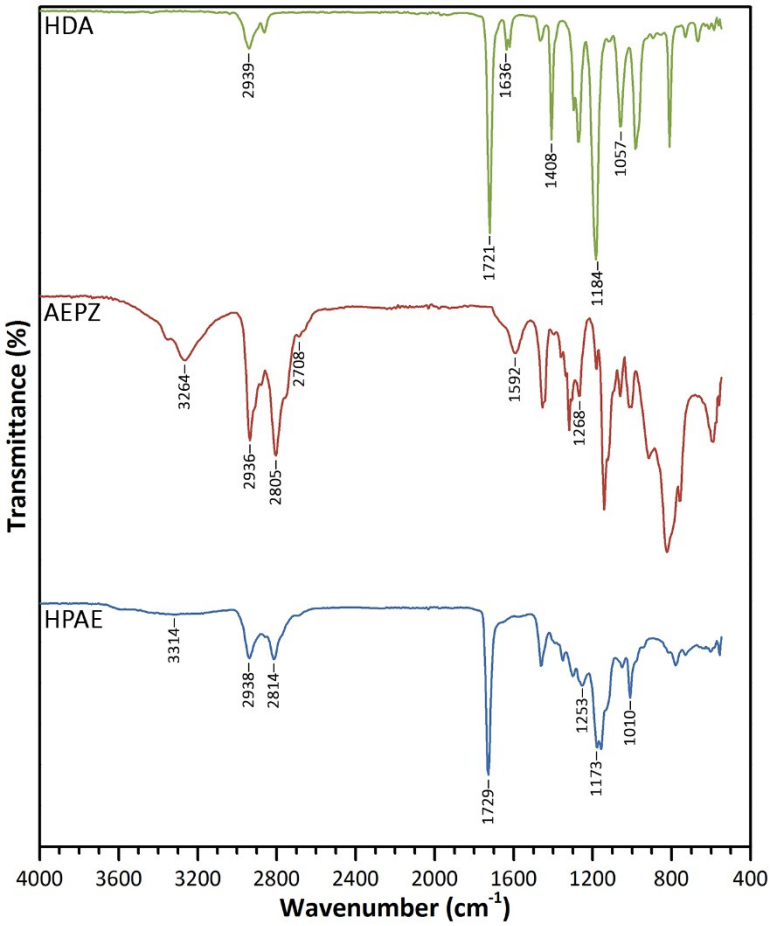

Figure S1*. FTIR spectra of HPAE and its starting materials.*

2.2. NMR spectra for LPAE, HPAE-C4Br, HPAE-C12Br and LPAE-C8Br

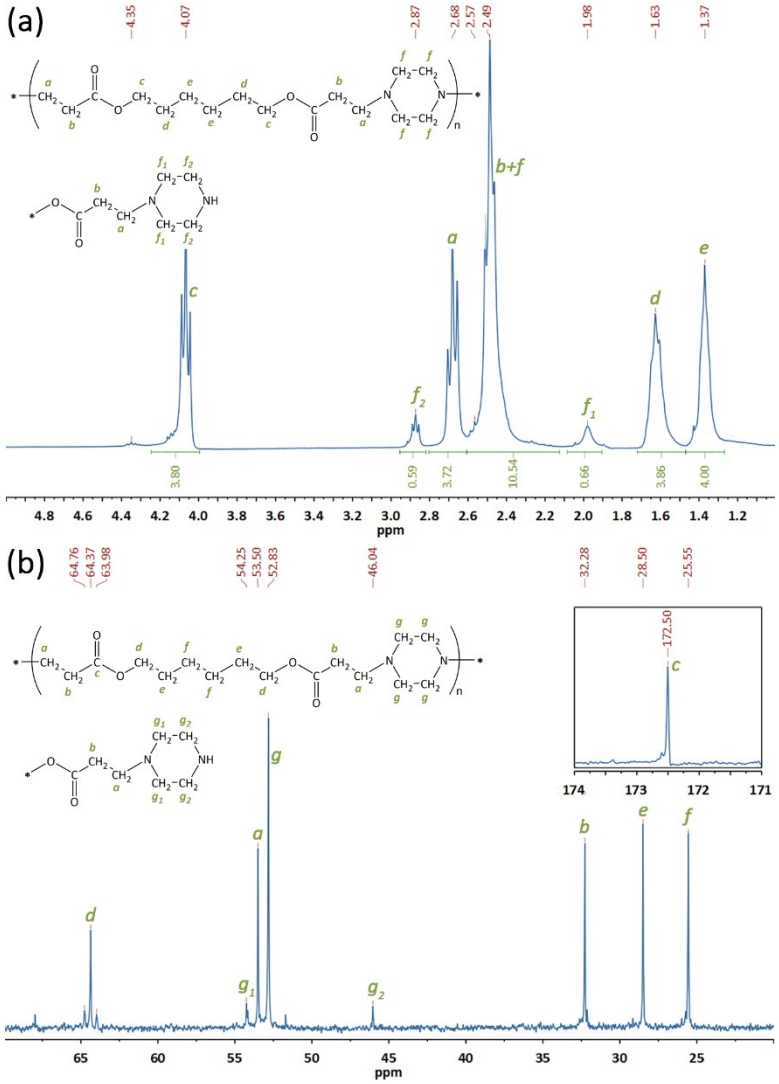

Figure S2. *^{1}H-NMR (a) and ^{13}C-NMR (b) spectra of LPAE in $CDCl_3$.*

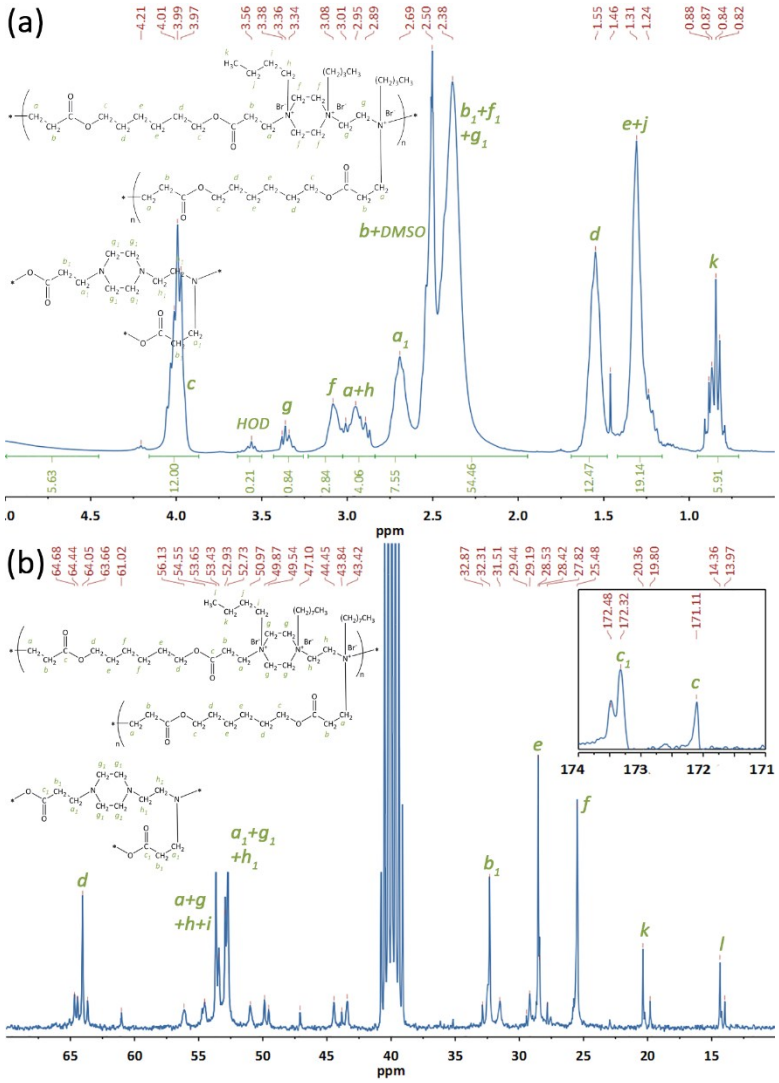

Figure S3. *^{1}H-NMR (a) and ^{13}C-NMR (b) spectra of HPAE-C4Br in DMSO-d_3.*

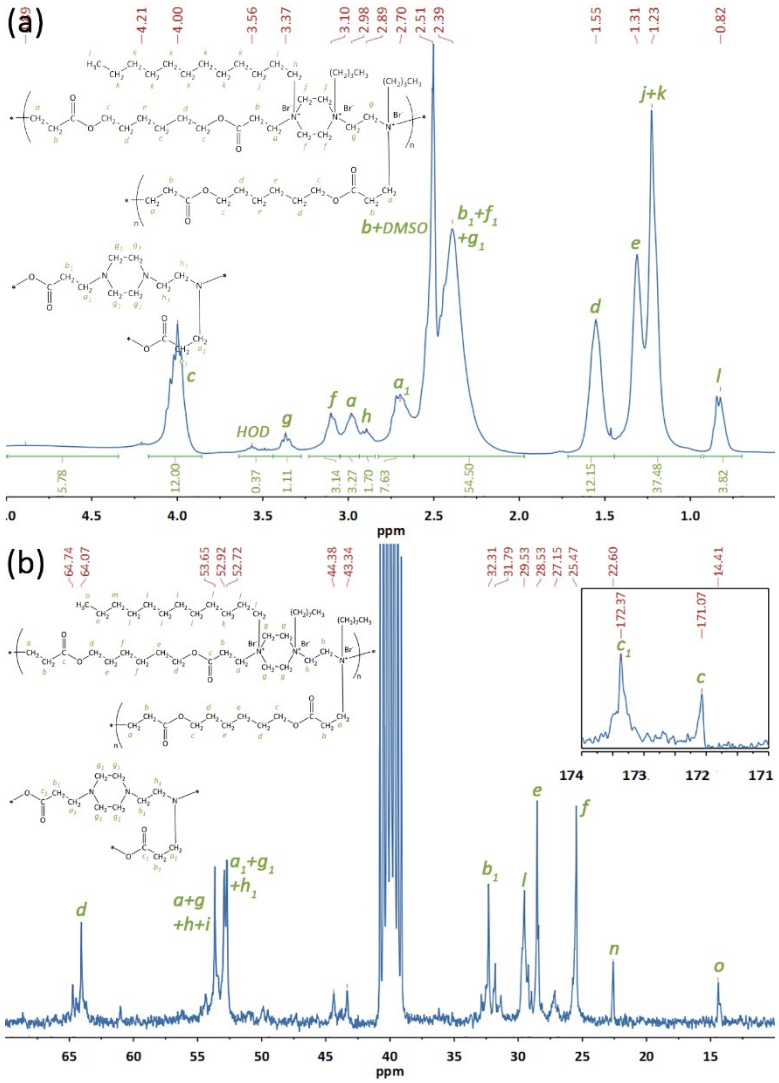

***Figure S4**. ^{1}H-NMR (a) and ^{13}C-NMR (b) spectra of HPAE-C12Br in DMSO-d_3.*

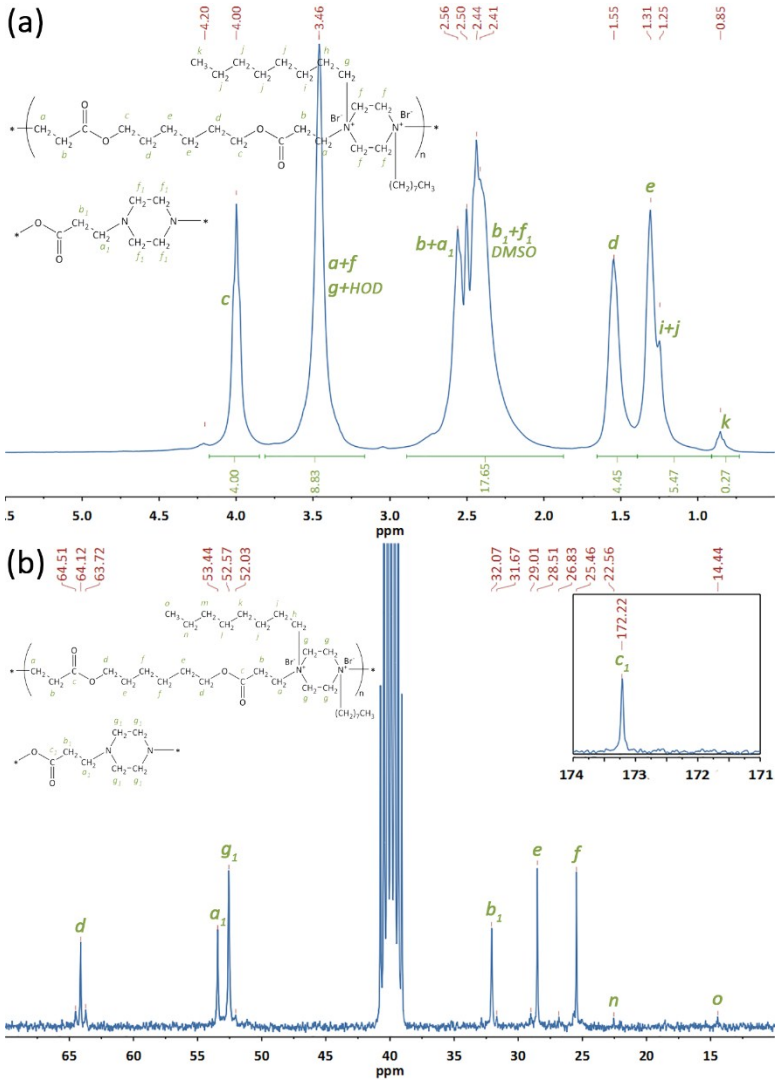

Figure S5. *^{1}H-NMR (a) and ^{13}C-NMR (b) spectra of LPAE-C8Br in DMSO-d_3.*

2.3. TGA and DTG curves for PAEs

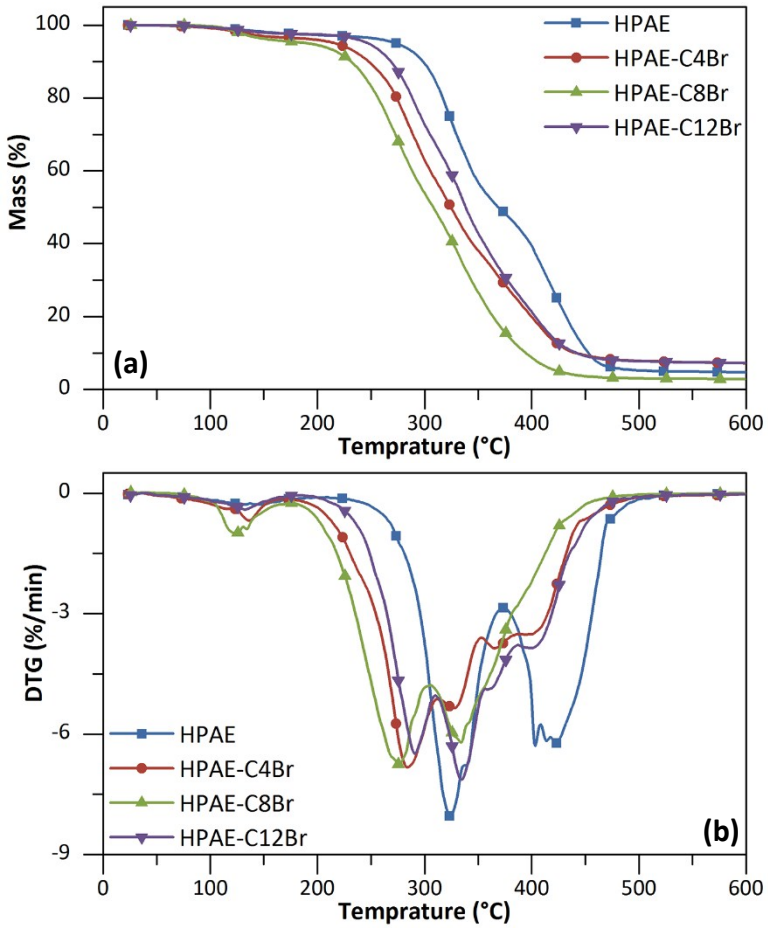

Figure S6. *TGA (a) and DTG (b) curves for HPAEs under N_2 atmosphere. The heating rate was 10 ^oC/min.*

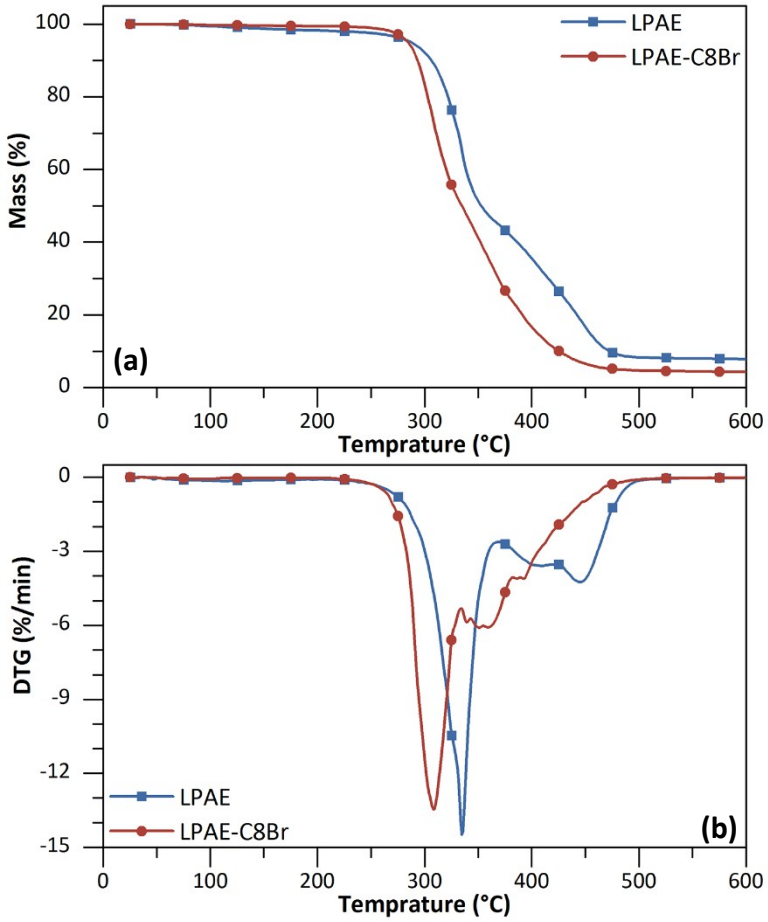

Figure S7. *TGA (a) and DTG (b) curves for LPAEs under N_2 atmosphere. The heating rate was 10 ^{o}C/min.*

2.4. DSC thermograms for PAEs

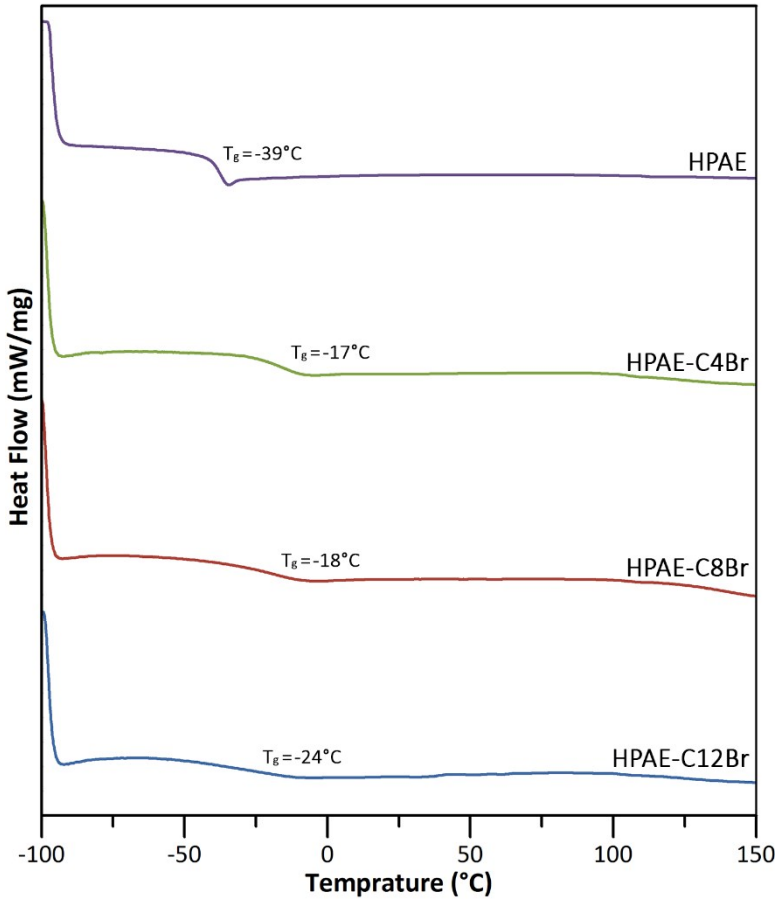

Figure S8. *DSC thermograms for HPAEs under N_2 atmosphere. The heating rate was 10 °C/min.*

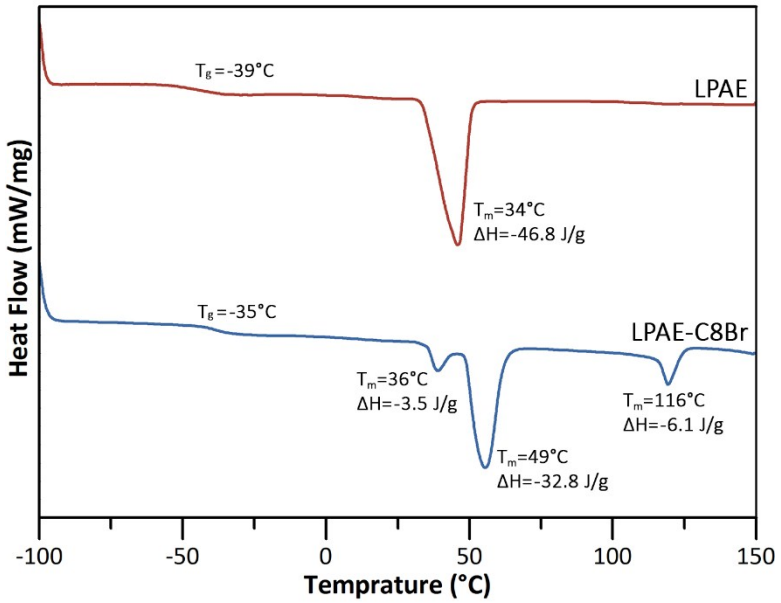

Figure S9. *DSC thermograms for LPAEs under N_2 atmosphere. The heating rate was 10 °C/min.*

2.5. TGA and DTG curves for PCL blends

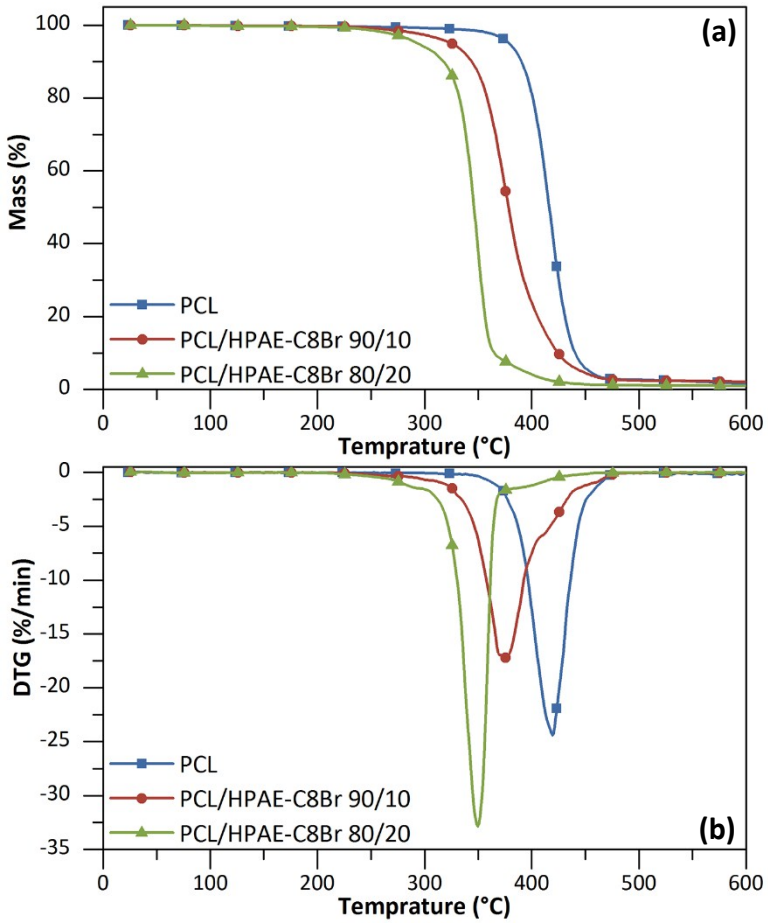

Figure S10. *TGA (a) and DTG (b) curves for PCL blends under N_2 atmosphere. The heating rate was 10 ^{o}C/min.*

2.6. DSC thermograms for PCL blends

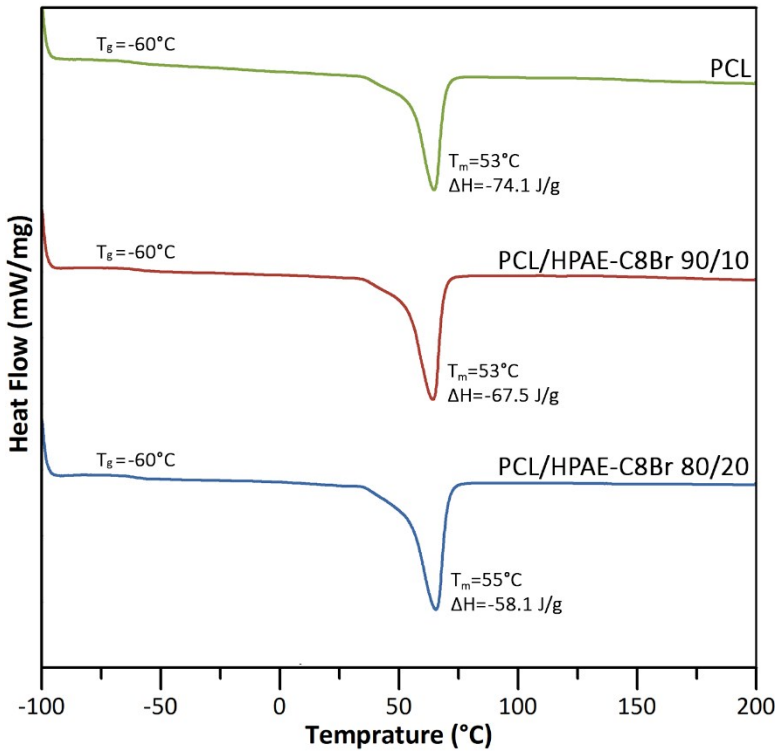

Figure S11. *DSC thermograms for PCL blends under N_2 atmosphere. The heating rate was 10 ^{o}C/min.*

2.7. *Contact angle of water droplets for PCL blends*

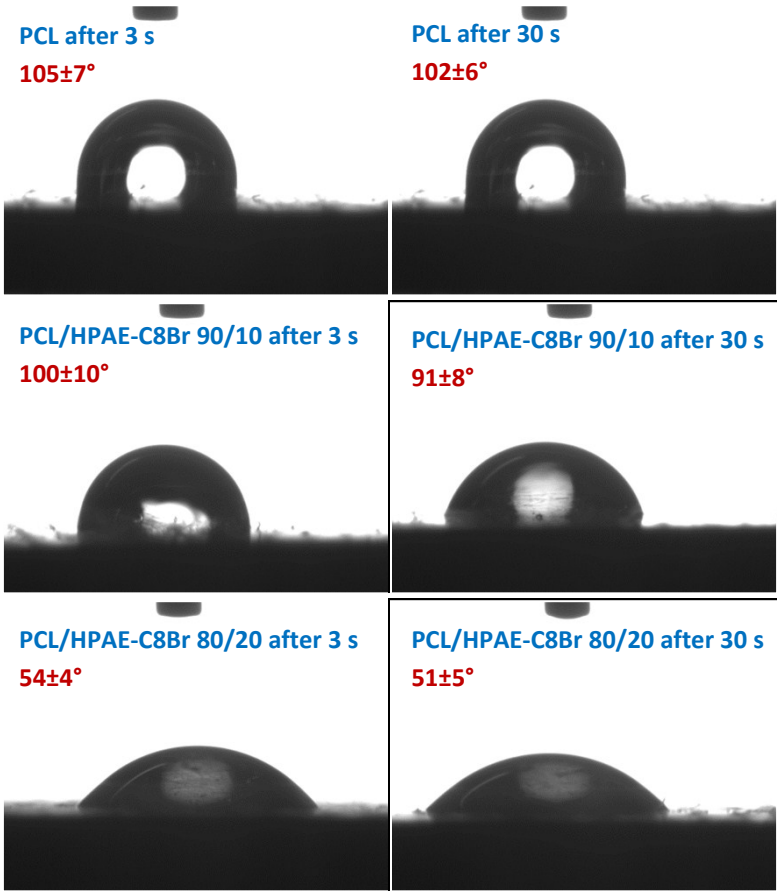

Figure S12. *Contact angle of water droplet (2 µL) on the surface of PCL blends.*

2.8. *Tensile curves for PCL blends*

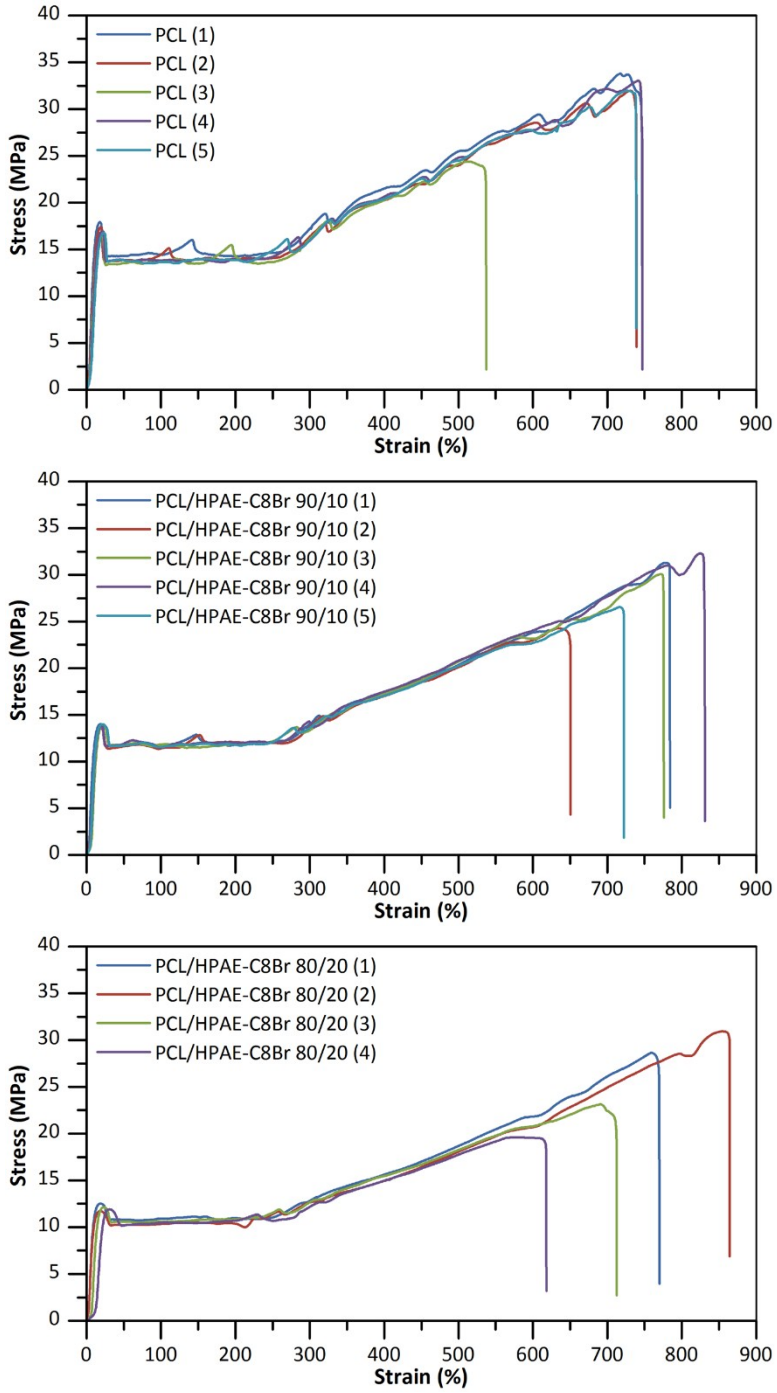

Figure S13. *Stress-strain curves for PCL blends. All specimens slipped out of grips before break.*

2.9. *Inhibition zones for QAS-PAEs*

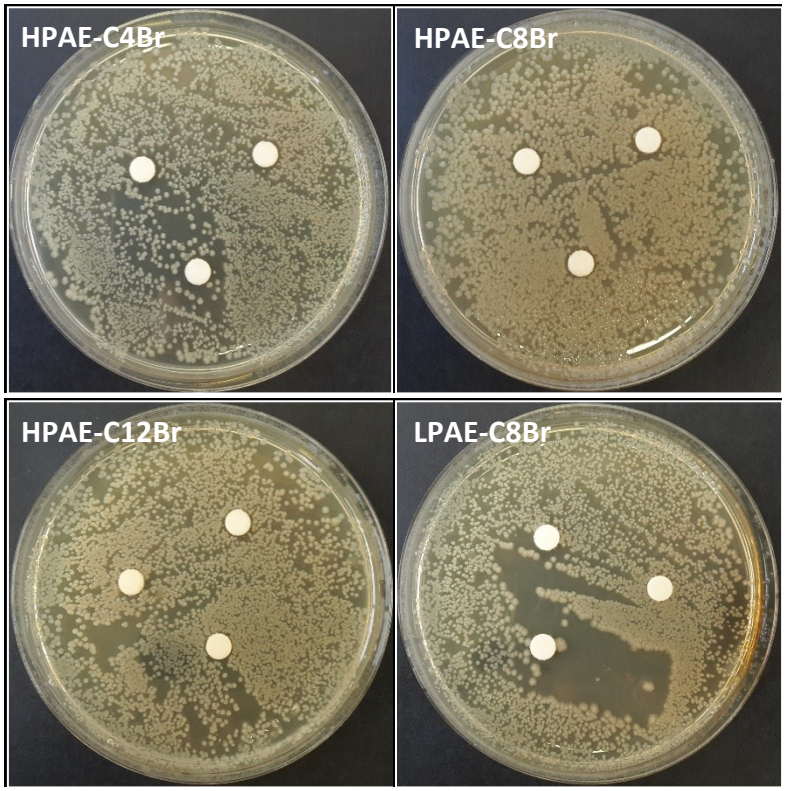

Figure S14. *Images for inhibition zone of QAS-PAEs against E. coli. Paper discs containing 100 µg of each sample were placed on nutrient agar plate seeded with 10^5 CFU of bacteria and incubated at 37°C overnight.*

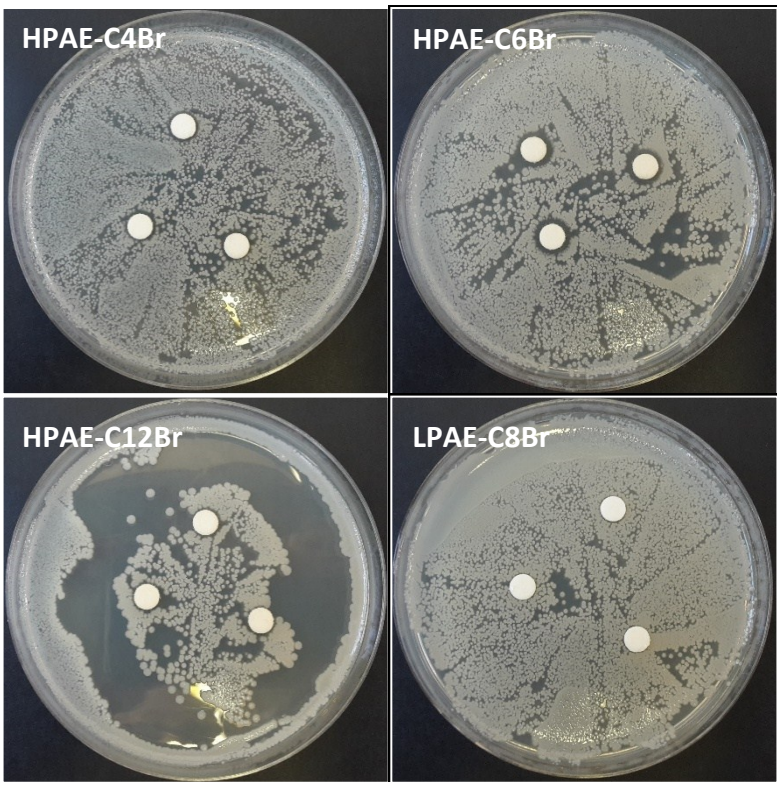

Figure S15. *Images for inhibition zone of the QAS-PAEs against B. subtilis. Discs containing 100 µg of each were sample placed on nutrient agar plate seeded with 10^5 CFU of bacteria and incubated at 37°C overnight.*

2.10. Inhibition zones for PCL blends

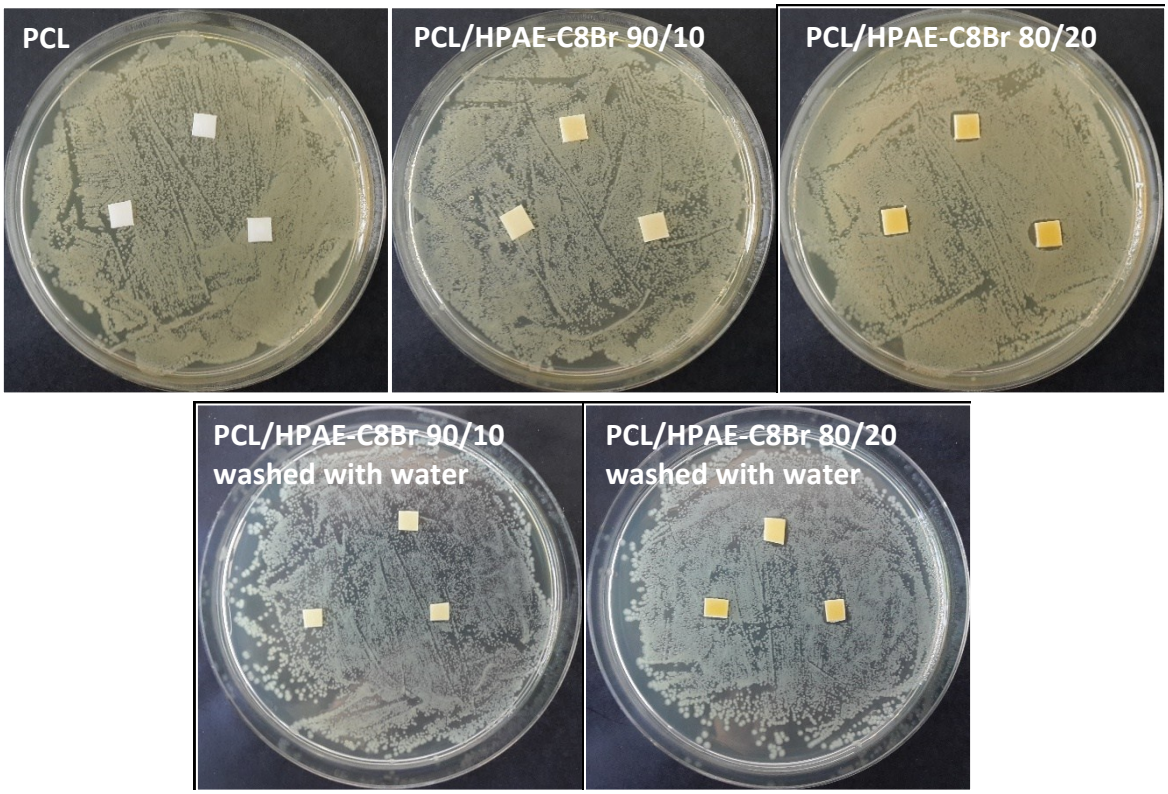

Figure S16. *Images for inhibition zone of PCL blends against E. coli. Images for quadrangular samples placed on nutrient agar plate seeded with 10^5 CFU of bacteria after incubation at 37°C overnight.*

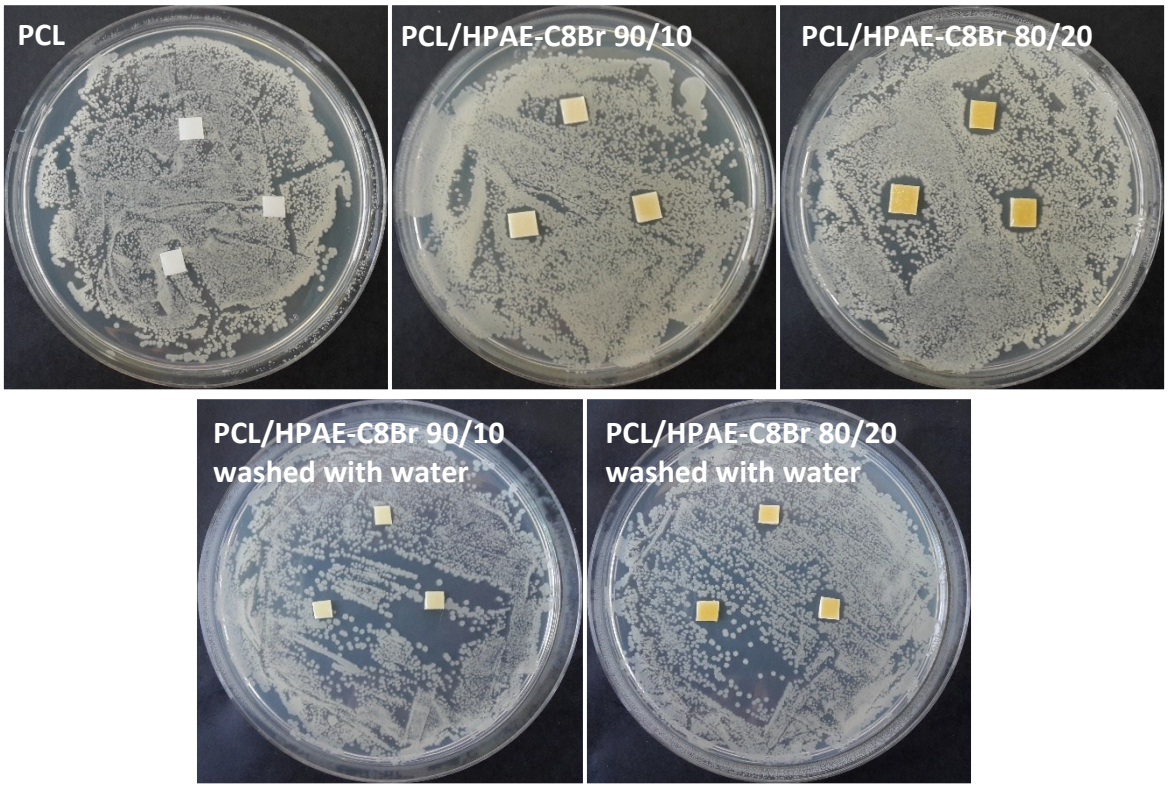

Figure S17. *Images for inhibition zone of PCL blends against B. subtilis. Images for quadrangular samples placed on nutrient agar plate seeded with 10^5 CFU of bacteria after incubation at 37°C overnight.*

2.11. EDX results for PCL blends

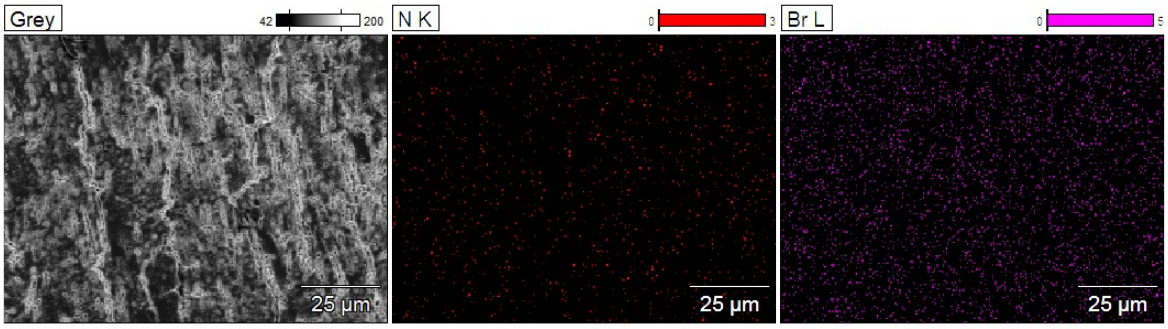

Figure S18. *Distribution map of nitrogen and bromide elements in PCL/HPAE-C8Br 80/20 after immersing in PBS medium (pH=7.4) containing Esterase (0.5 g/L) and NaN_3 (0.05 g/L) for 1 day.*

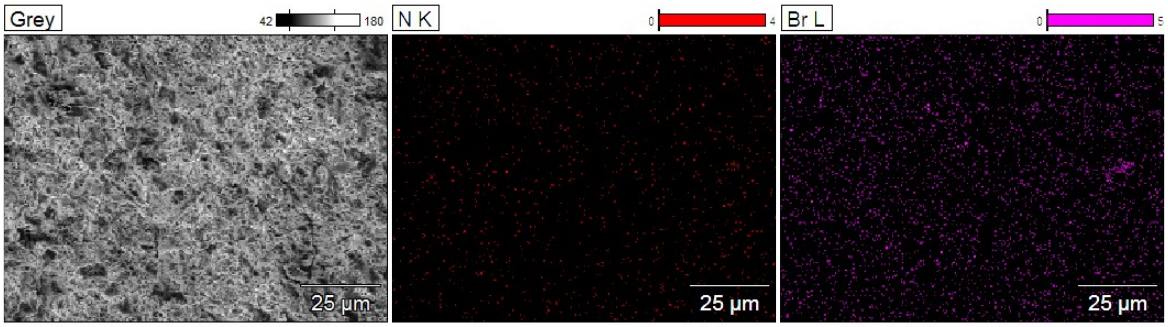

Figure S19. *Distribution map of nitrogen and bromide elements in PCL/HPAE-C8Br 80/20 after immersing in PBS medium (pH=7.4) containing Esterase (0.5 g/L) and NaN_3 (0.05 g/L) for 2 days.*

Table S1. *Elemental composition of PCL blends obtained from EDX analysis.*

Sample	Weight percent (%)			
	C	N	O	Br
PCL/HPAE-C8Br 90/10	50.69±0.29	3.08±0.61	38.68±0.57	3.57±0.29
PCL/HPAE-C8Br 80/20	53.60±0.25	7.05±1.02	36.70±0.25	4.87±0.13
PCL/HPAE-C8Br 80/20 after 1 day immersion	52.81±0.24	4.22±0.43	41.38±0.31	0.59±0.05
PCL/HPAE-C8Br 80/20 after 2 days immersion	49.64±0.30	7.71±1.20	41.40±0.75	0.63±0.17

3. References:

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