Coumarin-DPPO a new bio-based phosphorus additive for poly(lactic acid): Processing and flame retardant application

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Processing and flame retardant application

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Abstract

This study addresses very important aspects of the sustainable development of new flame retardant materials: (i) the preparation of a novel flame retardant (FR) additive from bio-resource, (ii) its use as FR additive in poly(lactic acid) (PLA) based composites and, (iii) the in vitro toxicity assessment of the FR. The synthesis of this bio-based FR additive was achieved through a phospha-Michael addition of diphenylphosphine oxide (DPPO) and the naturally occurring compound coumarin, yielding 4-(diphenylphosphoryl)chroman-2-one (CU-DPPO). The flame-retarded CUDPPO PLA composites were prepared either via conventional melt blending of commercially available PLA or through reactive extrusion (REX) of L-lactide. Characterization of the composites was performed through nuclear magnetic resonance (NMR), size exclusion chromatography (SEC) and thermogravimetric analysis (TGA). TGA analysis of the FR-PLA composites showed an increase in thermal stability, confirming the beneficial effect of the CU-DPPO additive. Direct insertion probe mass spectrometry (DIP-MS) analysis was used to gain insight on the mechanism of action of the FR additive, suggesting gas-phase activity. Rheological measurements exhibited the thermo-oxidative stabilizing effect in the modified PLA. The flame retardancy of the melt-blended PLA composites with CU-DPPO was investigated by limited oxygen index (LOI), cone calorimetry and vertical burning tests (UL 94 and BKZ-VB). Samples containing 10 wt.% of CU-DPPO showed improved fire performance with a limiting oxygen index (LOI) of 29 %, passing Swiss vertical burning test (BKZ-VB) and obtaining a V-0 rating in the UL 94 test. Moreover, a preliminary toxicity assessment of the CU-DPPO, carried out using an established in vitro platform, showing no adverse effects on cell viability.

Keywords

PLLA, Phosphorus flame retardant, Flame retardancy, Reactive extrusion

1   Introduction

PLA is a bio-based and bio-compostable polyester thermoplastic with environmentally friendly properties, it is derived from renewable resources such as cornstarch, tapioca roots, and sugarcane, making it a sustainable alternative to petroleum based polymers.[1]

This biodegradable polymer is mainly used in the production of packaging materials and medical devices;[2] it is also being increasingly used in the textile industry for making sustainable fabrics, in 3D printing for creating prototypes such as gears, car or electronic device parts.

PLA has excellent mechanical properties comparable to oil-based plastics such as poly(ethylene) (PE), poly(ethylene terephtalate) (PET) and poly(styrene) (PS) rendering it a viable alternative.[3,4]

However, like most thermoplastics, PLA is flammable; this in turn, limits its use and prevents its widespread application in areas of fire-safety such as in building infrastructure and transportation.[5]

In the last decade, advancements in flame resistance of PLA have been conducted, however most solutions employ inorganic fillers, such as aluminum hydroxide, ammonium polyphosphate and other e.g. melamine derivatives.[6-8] These solutions, still make use of high loading of additives which are typically sourced from petroleum-based compounds, thus making the PLA not fully bio-based.

Over the past two decades, there has been an emergence of phosphorus-based flame retardant (FR) for poly(lactic acid) (PLA) which include both inorganic and organic types.[9,10]

This work covers the synthesis of a novel bio-based organic phosphorus FR, which was obtained by reacting coumarin (a bio-based compound, member of the benzopyrone family, Figure 1) with diphenylphosphine oxide via a phospha-Michael addition. This novel additive, 4-(diphenylphosphoryl)chroman-2-one (CU-DPPO), shortly named CU-DPPO was studied as a potential FR for the production of PLA composites.

Firstly, the additive was tested for thermal stability, subsequently potential flame retardant application was assessed by melt blending with commercial PLA and testing the modified PLA for its fire performance. In a successive step, composites were prepared via reactive extrusion (REX) by a one-pot two-step process by directly in situ polymerization of (L)-lactide in an extruder and by the addition of CU-DPPO.
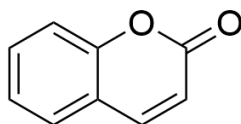

Figure 1: Chemical structure of coumarin.

REX involves in situ chemical reactions during the extrusion process to produce polymers or to functionalize them.[11] REX has the potential to produce FR polymers by adding reactive or non-reactive additives, in one-step compared to conventional polymer processing (such as melt blending) and chemical modification.[12]

This technique has been pioneered by Jacobsen et al. for PLA production and has gathered interest as a green approach for the manufacturing of PLA as no solvent is required to perform the reactions.[13]

An application case of this technique has been used in the work of Mincheva et al. where a phosphorylated PLA/poly(urethane) (PU) was produced through REX.

The authors were able to manufacture a flame-retarded composite with a reduced total heat release (THR) and heat release rate (HRR) by 35 and 36%, respectively, in addition to obtaining a V-0 classification in the UL 94 test.[14] There exists limited literature on the effect of organophosphorus additive in reactive extrusion process. Flame retardant functionalization of PLA in a one step process (i.e. REX) would help address sustainability requirements.

The flame retarded PLA composites in this work were fully characterized via nuclear magnetic resonance (NMR), thermogravimetric analysis (TGA) and size exclusion chromatography (SEC). The effects of FR on rheological properties of PLA blends were also studied.

2   Experimental section

2.1   Materials

Diphenylphosphine oxide (DPPO) was purchased by from Combiblocks, 1,1,3,3-tetramethylguanidine (TMG) was purchased from Acros Organics and both were used as received.

Tetrahydrofuran (THF), (L)-lactide, tin(II) 2-ethylhexanoate (Sn(oct)$_2$) were purchased from Sigma Aldrich. Commercial PLA (grade 3001D) with an average M$_w$ of 120 KDa, used in this study, was purchased from Natureworks LLC (USA). (L)-lactide was dried in a vacuum oven at 60 °C for 16 h prior to use.

Sn(oct)$_2$ was distilled under high vacuum and stored at -20 °C in a Schlenk tube. The Sn(oct)$_2$ 9% (vol/vol) solution was prepared as follows: a 10-mL, flame-dried, Schlenk tube connected to the gas manifold, was evacuated under vacuum and backfilled with N$_2$ three times.

Then under nitrogen (N$_2$), Sn(oct)$_2$ (0.9 mL) was cannula transferred in a 10-mL flamedried Schlenk tube and dry toluene (10 mL) was added.

Before processing, PLA pellets were dried in an air drying oven at 70 °C for at least 8 hours.

2.2   Synthesis of 4-(diphenylphosphoryl)chroman-2-one (CU-DPPO)
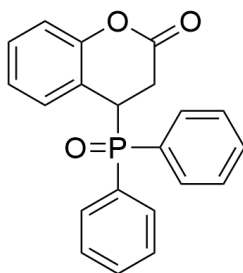

Figure 2: Chemical structure of 4-(diphenylphosphoryl)chroman-2-one (CU-DPPO).

A one liter, 3 neck round bottom flask was equipped with a Teflon-coated magnetic stir bar. The system was connected to a gas manifold and a bubbler was placed in the middle neck. THF (480 mL) was degassed for 5 minutes directly in the flask. DPPO (144.32 mmol, 29.18 g) was added

and after dissolution, TMG (20% mol, 3.6 mL) was added as base catalyst. After 5 minutes, coumarin (144.11 mmol, 21.06 g) was added portion-wise. The reaction was stirred for 2 h, a white solid slowly precipitated out. The mixture was filtered through a fritted glass funnel and the solid washed with fresh THF. The CU-DPPO was obtained as a white solid in 74% (144 mmol, 53,5 g) yield (Figure 2).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.89 - 7.80 (m, 2H), 7.66 - 7.49 (m, 6H), 7.42 (td, J = 7.7, 3.1 Hz, 2H), 7.28 - 7.19 (m, 1H), 7.01 (d, J = 8.1 Hz, 1H), 6.86 (t, J = 7.5 Hz, 1H), 6.63 (dt, J = 7.8, 1.9 Hz, 1H), 3.92 (ddd, J = 10.3, 7.9, 1.8 Hz, 1H), 3.20 (ddd, J = 16.7, 9.5, 1.7 Hz, 1H), 3.08 - 2.90 (m, 1H).

HRMS-ESI (m/z): found [M+H]$^+$ 349.0988, calculated C$_{21}$H$_{18}$O$_3$P requires 349.0988.

2.3   Polymer processing

2.3.1   Manufacturing of flame retardant composites from commercial PLA

Dried PLA was compounded with CU-DPPO additive in a twin-screw extruder (ZK 16 mm x 36 L/D, Collin Lab & Pilot Solutions, Germany) equipped with a gravimetric side feeding system to obtain pellets with 10 wt.% and 20 wt.% FR concentration. The temperature profiles of the twin-screw extruder were gradually increased from 180 °C (zone 1-3) to 185 °C (zone 4-6), corresponding to a steady-state measured melt temperature of ∼173 °C, except at the infeed zone (40 °C). A pelletizer (ThermoFisher Scientific VariCut, Germany) cut the extruded filament immediately to obtain pellets of homogeneous size distribution (pellet length = 3mm).

2.3.2   Reactive extrusion (REX)

Reactive extrusion polymers (samples names indicated with the prefix R-) were prepared in the microcompounder MC 15 HT (Xplore Instruments BV, The Netherlands) at a temperature of 185 °C and a screw speed of 100 rpm. The REX conditions were carefully selected to minimize thermomechanical polymer degradation at this stage. REX was carried out under continuous

argon flow (from the inlet into the microcompounder) to avoid any thermo-oxidative degradation caused by air. A blank REX-PLA (R-PLA) batch was prepared as follows: 20 g of the previously dried (L)-lactide was quickly weighed in a 50-mL glass beaker and covered with Parafilm™M. Then 0.5 mL of the Sn(oct)$_2$ 9% solution, was quickly added to the glass beaker and the mixture was brought immediately to the already pre-heated microcompounder. The reaction was monitored by the torque increase and the polymerization interrupted at a specific time, depending on the experiment. In the case of the preparation of PLA containing FR additives, either 2.23 g (equal to 10 wt.%) of CU-DPPO was added into the extruder after 15 minutes of the polymerization of (L)-lactide with the catalyst under the above mentioned protocol. Table 1 gives an overview of the composition of the starting materials of all the samples, including the additive contents and the total reaction times.

Table 1: Experimental conditions for reactive extrusion (REX) PLA specimen.

Sample Name	Additive	Additive (wt.%)	[Lactide]/[Sn]	Reaction temperature (°C)	Additive added (min)	Total reaction time (min)
R-PLA	-	-	1000	185	-	30
R-PLA-CU-DPPO	CU-DPPO	10	1000	185	15	30

2.3.3 Hot pressing as processing study

The materials from both commercial PLA compounding and reactive extrusion was granulated using a Hellweg M50/80 mechanical granulator (Hellweg Maschinenbau GmbH, Germany Germany). The resulting materials were dried in a vacuum oven at 80 °C for 18 hours, and subjected to hot-pressing in a Lindenberg Technics AG hydraulic press (Switzerland) at 185 °C, gradually increasing the pressure in stages (0 MPa for 2 min, 0.2 MPa for 2 min, 1.7 MPa for 2 min, 4.4 MPa for 2 min, and 10.9 MPa for 2 min) to produce rectangular plaques.

3 Analytics

3.1 Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) analyses were carried out. Around 2-4 mg of material were dissolved in 1 mL of 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP). The solution was injected via the autosampler, into a 1260 Infinity II High Temperature SEC System (Agilent Technologies, USA) equipped with a triple detector (refractive index detector, viscometer and light scattering detector). Measurements were performed with two Agilent PL HFIPgel (9 µm particle size) columnns (Agilent Technologies, USA) in series, after calibration with PS standards. The following measurement conditions are the selected: column temperature of 40 °C, flow of 1 mL/min of HFIP with as the solvent, and injection volume of 100 µL.

3.2 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was performed on a NETZSCH TG 209 F1 instrument (NETZSCH-Gerätebau GmbH, Selb, Germany) under N_2 (50 mL/min). Samples in the range 5-10 mg were placed in an open alumina crucible and were heated from 25 to 800 °C (heating rate of 10 °C/min).

3.3 Limiting Oxygen Index (LOI)

The limiting oxygen index (LOI) values of all plates were measured on an FTT oxygen index apparatus according to ASTM D2863-19. The specimen for the LOI test were of type III and the dimension were 150 x 10 x 1 mm^3.

3.4 Vertical burning tests and UL 94 test

Vertical burning tests (small scale fire tests) were performed according to the Swiss vertical burning test (BKZ-VB) standard as described in the literature.[15] Plate specimens were exposed, in

a vertical orientation, to a flame (45 °C) for 15 s and the burn length and burn time were measured. Most thermoplastic materials drip when exposed to fire and adequate heat. Thus, in the vertical burning tests, the dripping behavior of the specimens was also observed. The dimension of the specimen for the vertical burning tests was 160 x 60 x 0.6 mm^3.

The UL 94 vertical burning test was performed in a vertical burning chamber (Noselab ATS, Italy), the sample scale is 130 x 13 x 1 mm^3 according to ASTM D3801.

3.5   Cone calorimetry

Cone calorimetry (Fire Testing Technology, East Grinstead, London, UK) was performed with an irradiative heat flux of 35 kW/m^2 (ISO 5660 standard) on a specimens (100 × 100 × 3 mm^3) placed horizontally. Parameters such as heat release rate (HRR), peak of heat release rate (pHRR), total smoke production (TSP), total heat release (THR) were recorded.

3.6   Time-resolved frequency sweeps

Rheological measurements were carried out on hot-pressed films at 190 °C on an MCR 301 rotational rheometer (Anton Paar, Austria). The samples were carefully dried for 16 h at 80 °C under vacuum prior to be tested. A parallel-plate setting (plate diameter of 25mm and a gap of 1mm) was used for all the experiments. Samples, after being loaded, were melted for 2 min. A measurement of ten angular frequency sweeps (ω = 500 rad/s to 0.126 rad/s) was conducted at a constant strain of 1%. After each sweep, samples were relaxed for 60 s. A minimum of two time-resolved frequency sweeps (TRFS) measurements were performed for each sample.

3.7   Direct insertion probe mass spectrometry (DIP-MS)

Direct insertion probe mass spectrometry (DIP-MS) analysis was performed for a 1-2 µg sample amounts using a Thermo Scientific ISQ Series Single Quadrupole GC-MS apparatus equipped with a direct insertion probe (Waltham, MA, USA). The probe was heated from 30 °C to 450 °C at a

rate of 60 K/min and under high vacuum (10^{-6} mbar). The sample was kept at 450 °C for further 2 mins.

3.8 In vitro toxicity assessment of 4-(diphenylphosphoryl)chroman-2-one (CU-DPPO)

3.8.1 Cells

THP-1 (human monocytic) and A549 (human alveolar epithelial) cells, both obtained from ATCC, were cultured in RPMI-1640 medium (Sigma-Aldrich) supplemented with 10% fetal calf serum (FCS; Lonza), 0.2 mM L-glutamine (Gibco), and 1x penicillin/streptomycin (PS; Gibco). The cells were kept in culture in a humidified incubator at 37 °C and 5% CO_2. THP1 monocytes were differentiated into macrophages with 200 nM of phorbol-12-myristate-13-acetate (PMA; Sigma-Aldrich) in complete culture media for 72 h, followed by two washing steps with phosphate buffered saline (PBS; Sigma). For these experiments, only THP-1 differentiated into macrophages were used, but not monocytes. Only THP-1 differentiated into macrophages, but not monocytes, were used for these experiments. A 12.5 mM CUDPPO flame retardant stock solution in dimethyl sulfoxide (DMSO) was used for all in vitro toxicity tests. To prepare the flame retardant working solution for all the in vitro toxicity assessments, CU-DPPO was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 12.5 mM. Depending on the assay, this solution was further diluted in complete cell culture media (MTS assay and TNF-α ELISA) or in Hanks balanced salt solution (HBSS; DCF assay).

3.8.2 Cell viability assessment (MTS assay)

Cells were seeded in 96-wells plates (either 1x104 A549 cells/well or 8x104 THP-1 cells/well) and as pretreatment incubated for 24 h or 72 h prior treatment, respectively. CU-DPPO stock solutions were diluted in complete cell culture media at increasing concentrations (0- 100 µM) and added to the cells on the wells-plates. The treatment was carried out for 3 or 24 h at 37 °C and in an incubator with 5% CO_2. $CdSO_4$ served as a positive control agent. C, cell viability was

subsequently monitored using the CellTiter96®-AQueous One Solution Cell Proliferation Assay (Promega) according to the manufacturer's instructions. The assay contained MTS ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(4sulfophenyl)-2H-tetrazolium), which detects cell viability by indirectly measuring the activity of NAD(P)H-dependent dehydrogenase enzymes. In this process, the MTS reagent transforms into its formazan product only in metabolically active and living cells. Absorption was determined at 490 nm by using a Multimode plate reader (Mithras2, Berthold Technologies).

3.8.3   Microscopy

Before addition of the MTS reagent, CU-DPPO treated cells were imaged with an inverted brightfield microscope, using phase-contrast, a 10x objective (EC Plan-Neofluar) and ZEN software (Carl Zeiss Microscopy). Only untreated cells and the cells treated with the highest concentration of CU-DPPO were imaged.

3.8.4   Detection of reactive oxygen species (ROS) (DCF assay)

To determine if CU-DPPO can trigger the production of intracellular reactive oxygen species (ROS), the fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (H$_2$DCF-DA; Molecular Probes) was used. The cells were seeded in 96-wells plates at a density of 2x104 A549 cells or 8x104 THP-1 cells per well (24 or 72 h prior treatment, respectively). After two washing steps with PBS to remove all FCS, the cells were loaded with 50 µM H$_2$DCF-DA diluted in HBSS, followed by a 1 h incubation time at 37 °C. Subsequently, the cells were treated with increasing concentrations of CU-DPPO (0-100 µM in 100 µL/well in HBSS), and incubated at 37 °C for the indicated time points. The peroxynitrite-generating compound 3-morpholinosydnonimine (Sin-1; 50 µM in HBSS) served as positive control. Fluorescence intensity was measured every hour up to 4 h using a Multimode plate reader (Mithras2, Berthold Technologies) at an excitation wavelength of 485 nm and an emission wavelength of 528 nm.

3.8.5　　　　　Tumor necrosis factor alpha (TNF-α) detection

To exclude an inflammatory response of the cells to CU-DPPO, the detection of released TNF-α was done as with an ELISA assay. THP-1 monocytes were seeded in 96-wells plates (4x104 cells/well) and differentiated into macrophages. The cells were washed twice with warm PBS before adding increasing concentrations of CU-DPPO (0-100 µM in 100 µL/well of complete cell culture media). The cells were then incubated for 8 h at 37 °C. Lipopolysaccharide (LPS; Sigma) served as a positive control at concentrations of 10 and 100 ng/mL. The supernatant from the wells was harvested, centrifuged to remove all debris, and diluted 1:10 in complete media. Finally, a sandwich-ELISA (eBio-science) was performed following the manufacturer's instructions to quantify the total amount of TNF-α released by the cells.

4　　　　Results and discussion

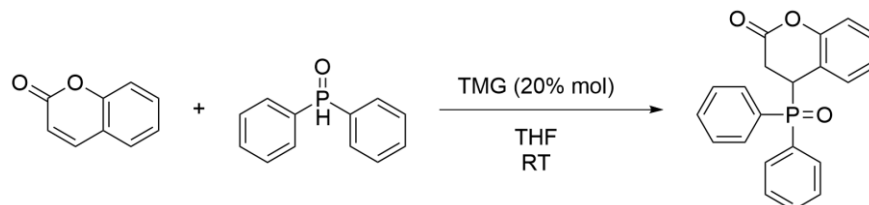## 4.1　　　　　Synthesis of CU-DPPO and its thermal properties

Figure 3: Reaction scheme of coumarin with diphenyl phosphine oxide to yield coumarinDPPO.

In designing the additive, a few desirable features were considered. In order to develop a bio-based phosphorus based fire retardant (FR), a bio-based monomer is required and preferably contain a P-C bond, which is hydrolytically very stable.

One of the most simple ways to obtain a P-C bond is through the phospha-Michael addition. The phospha-Michael addition requires a Michael donor (i.e. phosphorus monomer containing a P-H bond) and Michael acceptor (an α,β-unsaturated compound).[16,17]

Subsequently, for the bio-based core it was looked into the pool of naturally occurring compounds; coumarin was chosen as the candidate. Coumarin is derived from a natural source, such as cinnamon,[18] contains an aromatic ring which grants higher thermal stability[19] and the conjugated double bond for functionalization. Regarding the phosphorus-containing compound, diphenylphosphine oxide (DPPO) (a common phospha-Michael donor) was selected due to the presence of the phenyl rings directly attached to the phosphorus which grant higher thermal stability and, in addition, lead to an enhanced activity in the gas phase during combustion due to low degree of oxygen.[20-23]Phosphorus precursors containing P-O bonds such as phosphonates were avoided as phospha-transesterifcation reaction might occur with the unwanted release of alcohols during extrusion with PLA.

CU-DPPO has been previously reported by Miller and Stewart however, it was not possible to reproduce Lenker's synthesis method.[25,26] Thereby, a different route was chosen involving the base-catalyzed phospha-Michael addition (Figure 3).

Coumarin has a C=C double bond conjugated with an ester moiety; this has the possibility to undergo a phospha-Michael addition with phosphine oxide bearing a P-H bond.[16,27] The reaction was performed in the presence of catalytic amounts of a base, in our case 1,1,3,3-tetramethylguanidine (TMG) as reported by Simoni et al..[17,28] To the best of our knowledge there is no synthesis report of CU-DPPO in this manner; the strategy was chosen to avoid the use of silylating agents and anhydrous experimental conditions which requires a purification step through column chromatography.[29] In addition, no purification steps were required for the isolation of the compound, which is important for upscaling and industrial applications.

Regarding the thermal properties, the additive should not decompose in the processing window of PLA (which is between 190-200 °C). This was confirmed by TGA, which proved that CU-DPPO is stable well above 200 °C (Figure 4) having a 50% mass loss only above 300 °C.
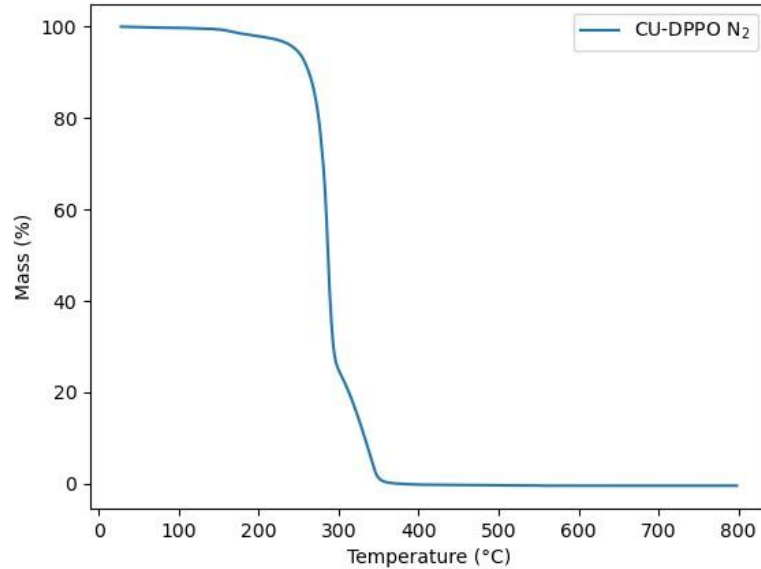
Figure 4: TGA curve of CU-DPPO in N_2.

4.2 CU-DPPO/PLA composite based on commercial PLA

The first experiments were thus conducted on the PLA-composites (produced as described in Section 2.3.1. Based on the phosphorus concentration of CU-DPPO, a 10 wt.% in the PLA blend would give roughly a 1% phosphorus content and 2% for a 20 wt.% in the final blend (as the CU-DPPO FR contains 8% phosphorus by weight).[30] This phosphorus concentration is typical for flame retarded polyesters.[31]

The molecular weight (M_w) of PLA samples were obtained by SEC analyses. As shown in Table 2 (Entry 1 and 3), the PLA with and without the additive have comparable M_w even after processing (Entry 2 and 4). This shows that, in this case, the incorporation of the additive has no detrimental effect on the M_w of the composites.

Table 2: M_w and dispersity (Đ) of compounded commercial PLA plates and PLA-CU-DPPO composite determined by SEC analysis.

Entry	Sample name	M_w (kg mol^{-1})	Đ$_1$
1	PLA	116.6	1.8
2	PLA-CU-DPPO 10%	113.8	1.7

4.2.1 Thermal decomposition of PLA-CU-DPPO composite

Thermal decomposition studies of commercial neat PLA and PLA containing CU-DPPO (PLA-CU-DPPO) compsites were both carried out under using TGA. As seen in Figure 5, the commercial neat PLA sample, show a one-step decomposition curve, while the sample containing CU-DPPO at 10 wt.% shows a slightly earlier decomposition temperature starting at 300 °C. Also, the composite at 20 wt.% of CU-DPPO displays earlier decomposition compared to blank PLA and the composite at 10 wt.%. This could be due to the catalyzed decomposition of PLA from the decomposition products of CU-DPPO. One has to mention here that this limited condensed phase action of CU-DPPO is not enough to increase the residue at higher temperatures, typically observed for phosphorus based FRs, suggesting that CU-DPPO might be acting primarily in the gas phase.[32,33]
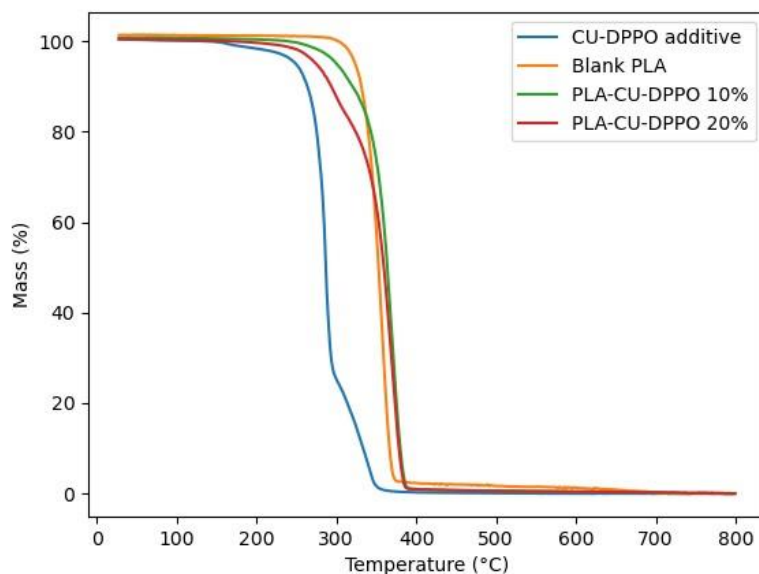
Figure 5: TGA curves of commercial PLA, PLA-CU-DPPO 10 wt. , PLA-CU-DPPO 20% composite and CU-DPPO additive (in N_2).

4.3   Flame retardant properties of PLA-CU-DPPO composite

PLA is a flammable material thus, its use in fire-safe applications is quite limited. Therefore, in order to investigate the fire behavior of PLA, small-scale fire tests were conducted on neat PLA plate samples via LOI, UL 94 and BKZ-VB tests (Table 4). The BKZ-VB test conducted on blank PLA resulted in no classification since the sample ignited immediately and the entire specimen burned completely due to its easy of flammability. The sample PLACU-DPPO burned for 1 second after the flame was removed. Dripping was observed without igniting the cotton below the specimen, which was considered as a pass in the BKZ-VB test. Pure PLA was readily flammable with a LOI value of 23.2% which is in agreement with literature values.[34,35] The LOI values of PLA-CU-DPPO 10 wt.% specimen increased to 29.5%, further loading 20 wt.% gave a value of 31.8% confirming its flame retardant behavior (Table 4).

The UL 94 test was also used to determine the efficiency of FRs since it is the most important and widely used method for screening the flammability of polymeric materials. It measures the resistance to sustain ignition by a small flame in a specific, well-defined scenario rather than evaluating a material property. UL 94 flame resistance takes into account factors such as the viscosity of the melt, which determines dripping behavior, thermal conductivity, specific heat, and the specimen geometry.[36] The relevant data obtained by UL 94 test are presented in Table 4.

Table 4: Fire-tests results of commercial PLA samples.

		UL 94			LOI	BKZ-VB				
Entry	Sample	Rating	Cotton ignition	t_1/t_2 (s)	Value	Rating	after flame (s)	burned (cm)	Dripping	cotton ignition
1	PLA	No classification	Yes	0/109	23.2 ± 0.1	Fail	-	-	Yes	Yes
2	PLA-CU-DPPO 10%	V-0	No	0/0	29.5 ± 0.1	Pass	1	6.8	Yes	No
3	PLA-CU-DPPO 20%	V-0	No	0/0	31.8 ± 0.3	-	-	-	-	-

In the UL 94 test, blank PLA obtained no classification as the sample caught fire and immediately the melting drops ignited the cotton below, after the second application of the flame the specimen burned up to the holding clamp displaying high melting while burning. For both CU-DPPO composites there was a self-extinguishing behavior while also heavy melting occurred (like for blank PLA), the flame needed to be adjusted by following the specimen, however the drops of the melting plastic did not ignite the cotton below. Both the composites containing 10 and 20 wt.% of CU-DPPO additive achieved V-0 classification.

Cone calorimetric test (CCT) is an effective method to further investigate the flame retardant performance and smoke production during the combustion of PLA composites.[15] The heat release rate (HRR), total heat release (THR) and total smoke production (TSP) are presented in Figure 6. From Figure 6a, PLA displays a sharp peak of heat release rate of 422 kW/m^2. The incorporation of CU-DPPO 10 wt.% contributed to a small reduction of pHRR by 13%. Increasing additional loading from 10 wt.% to 20 wt.% of CU-DPPO reduced the pHHR by 16%. The absence of char formation and the small reduction of pHHR suggests a gas-phase mode of action of the FR.

The time to ignition (TTI) slightly decreased as more CU-DPPO additive was incorporated, however the total heat release (THR) for the composite containing 10 and 20 wt.% was decreased by 27 and 39 %, respectively (Table 5).

Regarding the total smoke production (TSP), the blank PLA sample burns completely almost without smoke, however the addition of CU-DPPO into PLA promotes the smoke formation potentially due to the release of decomposition products from CU-DPPO, most likely phosphorus species which lead to incomplete combustion. This is also evinced by the increase in ratio of CO/CO_2 which suggests a gas-phase action of the additive.

The heat release rate (HRR) is used to calculate a parameter called maximum average rate of heat emission (MAHRE) between the start and end of the test, defined as the cumulative heat emission in the test period divided by the time.[37] The MAHRE (Figure 6b) for the samples containing CU-DPPO is lowered compared to blank PLA.

Table 5: Results from cone calorimetry tests for the investigated samples.

Sample	TTI (s)	pHRR (kW/m²)	THR (MJ/m²)	TSP (m²)	CO Yield (Kg/Kg)	CO₂ Yield (Kg/Kg)	Ratio CO/CO₂
PLA	80 ± 5	422 ± 46	63 ± 10	0.269 ± 0.108	0.018 ± 0.002	1.136 ± 0.002	0.02
PLA CU-DPPO 10%	74 ± 10	408 ± 22	46 ± 7	3.176 ± 0.625	0.052 ± 0.016	0.929 ± 0.127	0.06
PLA CU-DPPO 20%	59 ± 7	384 ± 3	39 ± 5	4.238 ± 0.538	0.071 ± 0.01	0.726 ± 0.086	0.1
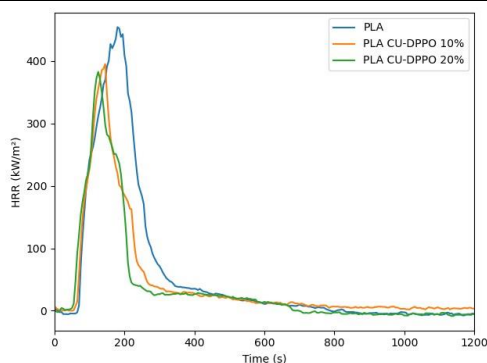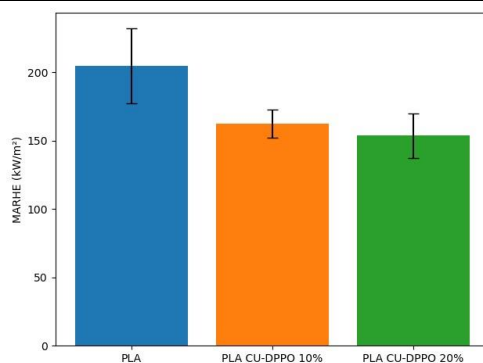
(a)   (b)

Figure 6: Cone calorimetric tests results of pure PLA and PLA-CU-DPPO composites.

After validating the CU-DPPO additive as a viable FR for PLA matrix, a novel set of experiments were undertaken to assess the proof of concept of utilizing CU-DPPO additive in the reactive extrusion (REX) process.

4.4 Reactive extrusion: screening and pre-validation

The first investigations in the attempt to manufacture reactive extrusion-PLA (R-PLA) composites was carried out by a one-pot step reaction in the a microcompounder where the polymerization of L-lactide is performed in the presence of a catalyst, as discussed in the literature.[13] In that frame, (L)-lactide, $Sn(oct)_2$ and CU-DPPO were introduced at the same time in the microcompounder.

Unfortunately, no polymerization of (L)-lactide was observed even after 30 min, as the presence of CU-DPPO was preventing the polymerization reaction. This could be explained by the interaction of the P=O with the Tin(II) metal center, which would deactivate the catalyst for ring-opening polymerization with the polymerization process.[38] Therefore, a onepot, two-step process was carried out with i) the initial polymerization of L-LA with $Sn(oct)_2$ and ii) the addition of CU-DPPO at a specific time, which yielded R-PLA-FR composites (Figure 7).
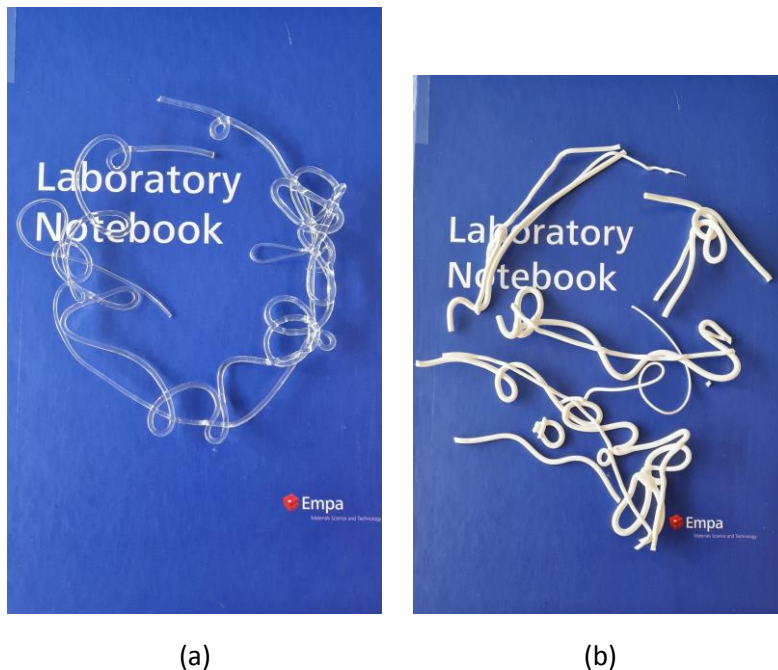

(a)   (b)

Figure 7: Extruded R-PLA blank (a) and R-PLA-CU-DPPO samples (b) prepared by reactive extrusion.

In order to understand the thermal stability, the decomposition behaviors and gain more understanding into the possible modes of action of the FRs, TGA studies of the composites obtained by reactive extrusion (REX) were carried out.

4.5 Thermal decomposition of REX PLA composites (R-PLA)

TGA of the PLA composites obtained by REX (R-PLA) show a different behavior; the blank R-PLA sample exhibits a two-step thermal decomposition (Figure 8) while the RPLA-CU-DPPO shows a three-step decomposition behavior. R-PLA degrades slowly in the first step, evidenced from the slope of the curve, from 120 °C to 250 °C with a weight loss of 21%.

In the second step, the decomposition continues from 260 °C to 280 °C with a weight loss of 98% and no residue at 290 °C. On the other hand, R-PLA-CU-DPPO initially degrades earlier between 100 and 250 °C (7% weight loss) and in the second step degrades between 250 and 330 °C (25% weight loss). However, in this case, there is a third decomposition step from 330 °C to 380 °C with a residual mass of 1.8% at 750 °C. According to the ^{1}H NMR of R-PLA, indicating residual lactide content of about ~9% is present in the blank R-PLA (Figure S14), this would explain the initial degradation step.[39] The second decomposition step, is assigned to the thermal decomposition of the PLA matrix for the R-PLA (sample without the FR) as described by McNeill and Leiper.[40] The decomposition of blank PLA results from inter- and intra-molecular reactions such as transesterification and unzipping of the polymer.[41]

In the case of R-PLA-CU-DPPO, the degradation is also attributed also to the degradation of the residual monomer in the PLA matrix, however the CU-DPPO additive is clearly enhancing the thermal properties of the composite. The difference of 87 °C of the onset temperature difference, compared to the neat R-PLA, shows that the presence of CU-DPPO stabilizes the polymer thermally.

The third step is also attributed to the thermal decomposition of the PLA matrix suggesting that after the complete degradation of CU-DPPO in the polymer matrix, depolymerization of PLA

takes place, however this process is delayed compared to R-PLA. This effect is also evinced in the derivative thermogravimetry (DTG) curve (Figure S23), where the peak corresponding to the maximum weight loss shifts from 268 (for R-PLA) to 356 °C (for R-PLA-CU-DPPO).
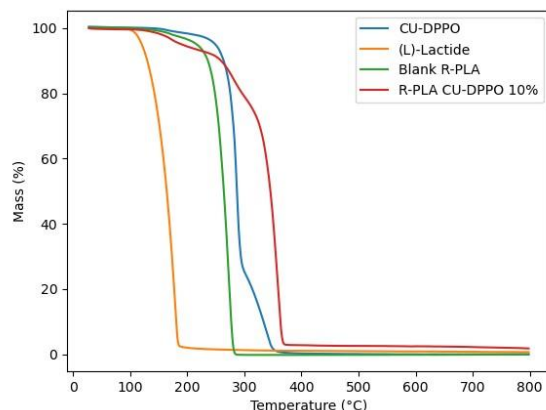

Figure 8: TGA curves of CU-DPPO additive, (L)-Lactide, REX-PLA (R-PLA) and R-PLACU-DPPO 10 wt.% (in N_2).

4.5.1 Investigation of CU-DPPO FR mode of action through DIP-MS measurements and NMR analyses

DIP-MS is a pyrolysis-mass spectrometry type technique, which allows the study of the decomposition products of a polymer matrix by thermally decomposing and volatilizing the components through a gradual increase in temperature generating a product distribution of ionized species. During DIP pyrolysis, thermal degradation takes place within the mass spectrometer, where pyrolyzates promptly move from the heating zone to the source region for ionization. This process eradicates the chance of secondary and condensation reactions. Moreover, the high vacuum within the mass spectrometer promotes vaporization, facilitating the analysis of pyrolyzates with higher molecular masses. The rapid detection capability of the mass spectrometers allows for the identification of unstable thermal degradation products. The total ion current (TIC) chromatogram represents all of the charged species which are being detected at each point in time throughout the analysis, while the extracted ion current (EIC) represents

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the reconstructed chromatogram, of a species having a specific m/z value (that can be chosen) given from the entire data set of the analysis.

Consequently, a more comprehensive overview of released species during the pyrolysis can be attained.[42] In order to understand the underlying decomposition of the PLA polymer and mechanism of action of the FR, more specifically in the gas-phase, DIP-MS was carried on both R-PLA-CU-DPPO and R-PLA for comparison.[43] Figure 9a and 9b show the chromatogram of the total and extracted ion current (TIC and EIC) for R-PLA (Table 6, entry 2) and R-PLA-CU-DPPO (Table 6, entry 4), respectively.

For blank R-PLA, in the controlled heating environment, starting at 5.5 min, volatilization of ionized species begins as shown in the TIC (Figure 9a); this corresponds to the initial decomposition, which is taking place at 330 °C. The main fragments released at the beginning of the decomposition are acetaldehyde (44 m/z) and methyl ketene (56 m/z) which are formed from the breakdown of the PLA backbone (Figure S18). In addition, a small peak corresponding to 144 m/z (7%) is present, may be assigned to the volatilization of the lactide monomer.[40,44]

As shown in Figure 9a, the peak at 6.8 min (408 °C) indicates the maximum volatilization rate corresponding to a m/z of 145 (Figure S19) which can be identified as the decomposition fragment of PLA's main chain clearly indicating that depolymerization is occurring (Figure 9a, EIC). Once the peak has been reached, no more species are detected indicating that no residue is present at 7.1 min, at a temperature of 426 °C, showing that blank PLA decomposes in a one-step process.
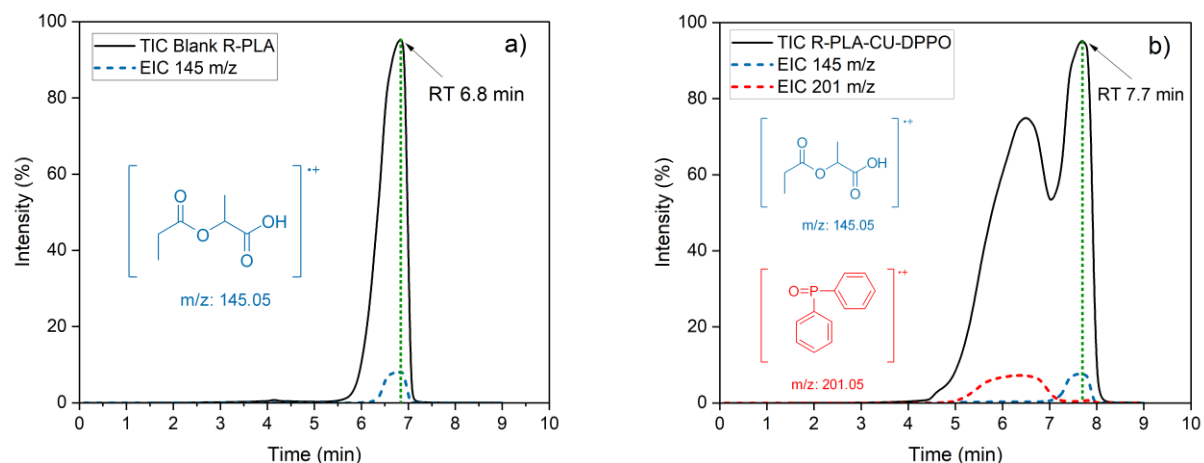
Figure 9: DIP-MS TIC and EIC chromatogram of a) R-PLA and b) R-PLA-CU-DPPO.

Conversely, for R-PLA-CU-DPPO, the TIC chromatogram shows a different curve pointing to a two-step decomposition process (Figure 9b).

The first degradation products arise earlier (compared to blank R-PLA) at 4 min to around 7 min (Figure 9b and S20). This is attributed to the decomposition of the CU-DPPO additive PLA matrix showing a degradation behavior earlier compared to blank PLA, clearly showing a delaying effect on the decomposition of the polymer backbone. In the EIC corresponding to 201 m/z, the species belonging to the DPPO fragment is detected starting from 5 min up to 7 min (300 to 420 °C, respectively). This evidence is also corroborated in the mass spectrum (Figure S21) showing the molecular ion peak at 348 m/z and the fragmentation pattern shows the major ion at 201 m/z corresponding to DPPO (as schematically proposed in Figure 10).

At 7 min, where the two TIC peaks overlap (Figure 9b), the intensity of the DPPO fragment at 201 m/z is reduced compared to the main decomposition products of PLA matrix observed at 145 m/z (Figure 9b EIC and Figure S21), thus indicating that CU-DPPO is almost totally consumed early in the decomposition process. Subsequently, at 7.7 min (450 °C), when the second TIC peak reaches its maximum and, when all the CU-DPPO has decomposed and/or volatilized, the decomposition of PLA starts via the same mechanism already described earlier above with the

characteristic main signal at 145 m/z (Figure 9b and Figure S22). As observed in the TIC (Figure 9b), the start of the decomposition of the PLA is shifted to 7 min instead of the 4 min determined for the blank PLA (Figure 9a), indicating that CU-DPPO acts as a retardant of the decomposition of PLA and supposedly is working in the gas phase during combustion.
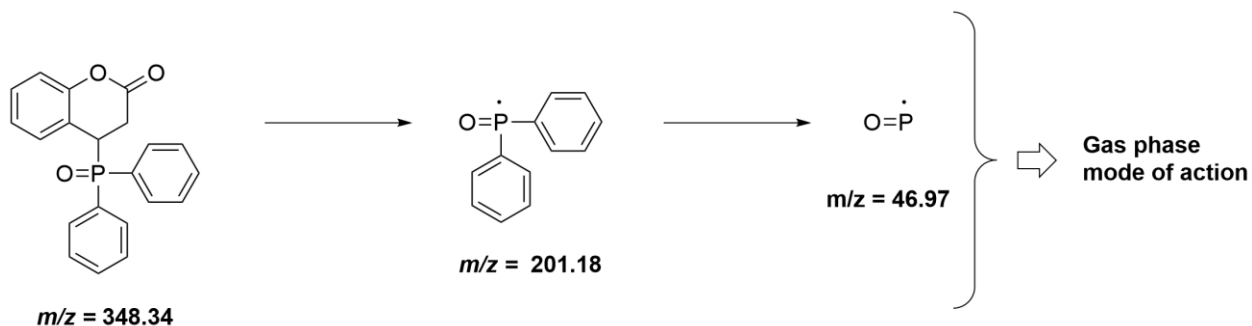

Figure 10: Proposed thermal decomposition pathway for CU-DPPO based on DIP-MS analysis.

NMR analysis was carried out in order to better understand the possible interaction of CU-DPPO and polymer matrix and its role in increasing the thermal stability. Three NMR experiments were conducted on the REX composite (Figures S15 and S16) which was thermally treated in the TGA up until 330 °C (corresponding to the second decomposition step, supposedly belonging to the decomposition of CU-DPPO). NMR spectra of the thermally treated R-PLA-CU-DPPO were acquired both in proton and phosphorus.

In the ^{1}H NMR spectrum of the heated material it is evident that the DPPO fragment is no more present in its original form after the thermal treatment as the signals positions of resonances in the aromatic chemical shift region is greatly heavily changed (8.0-7.3 ppm) and the appearance of new signals in the chemical shift region assigned to olefins suggests that an elimination type of reaction may have occurred with CU-DPPO (Figure S15).

As seen in the ^{31}P NMR, for the thermally treated R-PLA-CU-DPPO, two new peaks appears (at 35 and 34 ppm) compared to the CU-DPPO monomer (at 31 ppm) (Figure S16). This may indicate that the CU-DPPO is partly degraded and new species are formed, however, it was not possible to determine the actual structure of the decomposition products with only this experiment. It is

likely that CU-DPPO reacts with the decomposition products of PLA to form new phosphorus species in the condensed phase.

A diffusion-ordered NMR (DOSY NMR) experiment on an extruded sample of R-PLACU-DPPO which was also thermally treated, was used to investigate whether the CU-DPPO molecule could be present bound within the PLA polymer matrix or whether it is more likely to be present as a more loose copolymer without a tight bond to the PLA backbone. NMR measurements of the DOSY type can be used to determine the diffusion coefficient of a species in a solution.

For example, if the CU-DPPO units or their decomposition products were covalently bound to the PLA matrix, only one diffusion coefficient could be observed for all chemical species. As observed in the 2D plot, the ^{1}H NMR signals arising from the additive's species (labeled in the red box, Figure S17), have a slightly lower diffusion constant compared to the signals belonging to PLA; this clearly indicates that there is no incorporation of the additive into the PLA backbone during the thermal decomposition process.

4.6   Melt stability

It is important to study the effects of the FR additive on the stability of PLA melt since it significantly impacts the processability, recyclability, and overall stability. Rheology is sensitive to molecular changes since it assesses the mobility of the (entire or partial) polymer chain. TRFS rheological tool allows to study the chemical changes of PLA in molten state by multiple consecutive frequency sweeps. The complex viscosity of R-PLA and R-PLACU-DPPO with TRFS rheological experiments are presented in Figure 11.

The little difference in rheological behavior in air and N_2, irrespective whether CU-DPPO is present or not, suggests that both compounds up to 190 °C are not as particularly sensitive to oxidation as at elevated temperatures. R-PLA shows a decay in viscosity over time (loss of ∽46% of its initial value), which is a typical indication of the thermal decomposition of the polymer. It

behaves like Newtonian liquids since applied frequency shows no effects on the complex viscosity.

On the other hand, the viscosity of R-PLA-CU-DPPO slightly increases in the first 30 min and eventually reaches a plateau. The initial increase can be explained as continued reactions of the residual lactide in the R-PLA-CU-DPPO compound.

As rheological tests continue, CU-DPPO hinders the decomposition in PLA and stabilizes the polymer melt. Lower frequency suggests a longer relaxation time, which pictures the flow behavior of polymer chains. The complex viscosity of R-PLA-CU-DPPO has different values demonstrating a higher viscosity at lower frequencies, which is a characteristic behavior of composites with non-Newtonian viscoelastic behavior.[45]
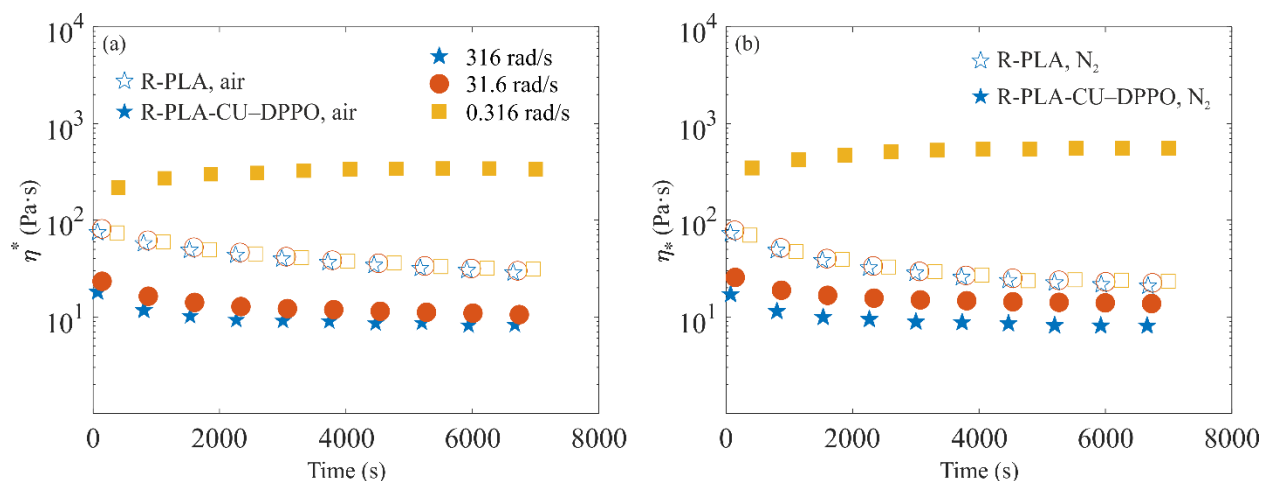
Figure 11: Time-dependent rheological behavior of R-PLA (hollow symbols) and R-PLACU-DPPO (solid symbols) at 190 °C (a) in air and (b) under N_2. Different symbols refer to different angular frequencies.

4.7 Process stabilization of CU-DPPO on REX composites

In order to assess the stabilization effect of the FR during the polymerization and the processing of the formed composites, a few approaches were explored. The initial goal was to obtain a flame retarded PLA in a one-step process, i.e. by performing the ROP by pre-mixing the lactide, catalyst and FR.

However, under these experimental conditions, it was not possible to obtain any polymer, as the polymerization was not initiated (as described in Section 2.3.2). Using an alternative strategy, the polymerization was started in a microcompounder, as was the case for the R-PLA blank. This time, however, the CU-DPPO additive was added after polymerization at a specific time depending on the experiment. After the addition of the additive, the mixture was further processed for 10 minutes before the polymer was extruded. Following the above mentioned procedure, two sets of R-PLA were prepared: a blank R-PLA with a total reaction time of 30 min and a R-PLA with CU-DPPO added at 15 min with a total reaction time of 30 min (R-PLA-CU-DPPO). In order to study the effect of the FR during the REX process, SEC measurements were performed for samples taken at each step of the processing from the first milling process to the plate manufacturing, the results are shown in Table 6.

Table 6: Sample names, molecular weights and polydispersity indices of R-PLA-CU-DPPO (prepared by addition of 10 wt.% CU-DPPO) compared and R-PLA for each dedicated processing steps.

Entry	Sample Name	M_w (kg mol^{-1})	Đ
Blank PLA 1			
	REX PLA milled	105.8	1.6
2	REX PLA plate	57.0	1.6
CU-DPPO 10%			
3	REX PLA milled	89.0	1.5
4	REX PLA plate	64.9	1.6

As seen from Table 6, the R-PLA-CU-DPPO samples initially had an initial lower molecular weights as the corresponding R-PLA material. However, in comparison during successive each processing steps, the reduction in molecular weight is somewhat higher for the blank R-PLA samples.

This study reveals the possibility that samples containing CU-DPPO in 10 wt.% are less prone to decomposition during the thermal processing steps which would indicate a higher thermo-oxidative stability of the mixture. This characteristic could be interesting for mechanical recycliability of the composites and will be further addressed in later studies.[46]

4.8 In vitro toxicity assessment of CU-DPPO additive

Toxicity assessment of CU-DPPO were performed with three, in vitro, experimental approaches in human cells: a viability assay to quantify cell death caused by CU-DPPO, a ROS assay to determine cellular stress, and detection of the pro-inflammatory cytokine TNF-α as a measure of inflammation. This in vitro toxicity assessment was carried out as described in our previous publication and other groups for other flame retardant additives.[47,48] Two model cell lines were used: human lung epithelial A549 cells and macrophages derived from the monocytic cell line THP-1.

Cell viability was assessed after 3 and 24 h of CU-DPPO treatment using the MTS assay. As seen in Figure 12 (A-B), CU-DPPO did not affect cell viability of both A549 cells and THP-1 macrophages at any of the tested concentrations and treatment times. On the other hand, the positive control $CdSO_4$ reduced cell viability in both cell types in a dose-dependent manner in the same concentration range (Figure 12 C-D).
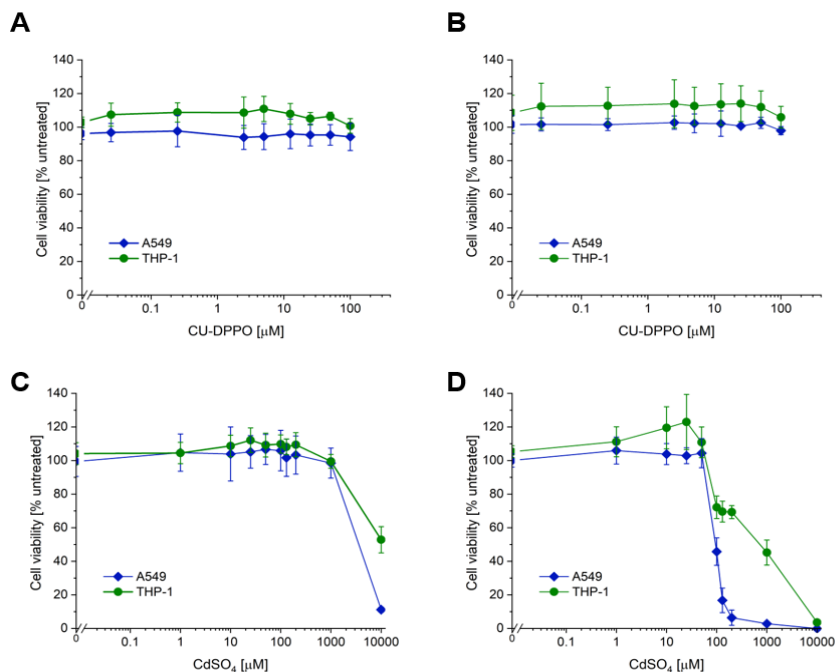
Figure 12: Impact of CU-DPPO on cell viability of two human cell types. Lung epithelial cells (A549 cell line) and THP-1 derived macrophages were treated with an increasing concentration of CU-

DPPO for (A) 3 h and (B) 24 h. Cell viability was then measured using an MTS assay. CdSO$_4$ was used as a positive control also at (C) 3 h and (D) 24 h of treatment. The values represent the mean and standard deviations of three independent experiments. The data were normalized to the untreated control samples.

The effect on cell viability was also clearly observed by changes in cellular morphology induced by CdSO$_4$ but not by CU-DPPO (brigthfield microscopy images Figure S25). Besides cell death, CU-DPPO could also cause other adverse effects at sub-lethal conditions. Hence, the production of intracellular was quantified ROS and the release of the pro-inflammatory cytokine TNF-α, measurements of acute cytotoxicity in vitro.

Up to 100 µM, CU-DPPO did not increase the level of ROS released by both cell types, compared to the solvent control (Figure 13A and Figure S26). The positive control Sin-1 induced a significant increase in ROS production in A549 and THP-1 macrophages.

A similar non-cytotoxic effect of CU-DPPO was observed when measuring TNF-α (Figure 13B), an indicator of an inflammatory response.

In THP-1 macrophages, the treatment with CU-DPPO did not induce the release of TNFα. In this case, LPS was used as a positive control. From these results, it can be concluded that CU-DPPO does not induce an acute cytotoxic effect at the evaluated concentrations. It is important to mention that CU-DPPO has a reduced solubility and stability in DMSO.
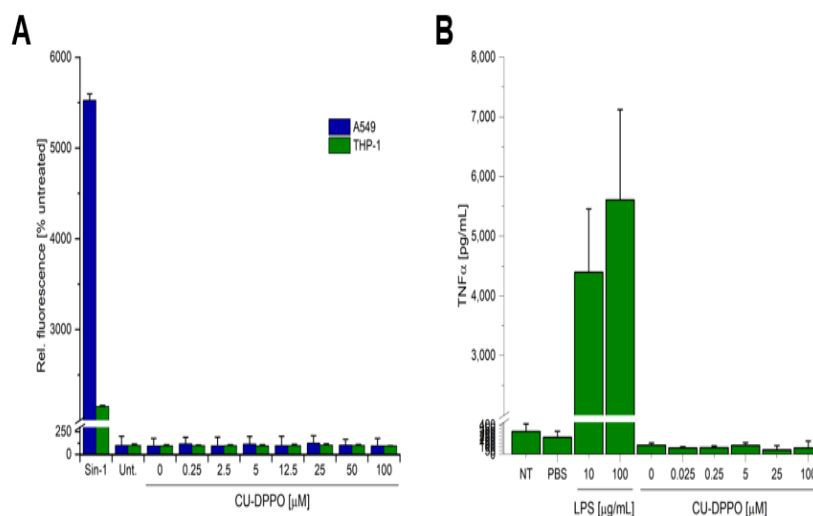
Figure 13: CU-DPPO did not cause acute cytotoxicity at sub-lethal concentrations in vitro. (A) ROS overproduction was assessed with a DCF assay in A549 and THP-1 macro-phages upon CU-DPPO treatment (0-100 µM). Shown are the results for a 2 h treatment (the results for 1, 3 and 4 h are in Figure S26). Sin-1 (50 mM) was used as the positive control. The data is shown as the percentage with respect to the untreated con-trol (Unt.). (B) Detection of the pro-inflammatory cytokine TNF-α was done in THP-1 macrophages, which were treated for 8 h with the indicated concentrations of CU-DPPO or LPS as a positive control. The amount of TNF-α in the supernatant was deter-mined with an ELISA assay. The untreated control (Unt.) means just cell culture media was used. The "0 µM" condition corresponds to the dimethyl sulfoxide (DMSO) control diluted in cell culture media. Data shown in A-B represent the mean and corresponding standard deviations of three independent experiments.

CU-DPPO is soluble in DMSO up to 12.5 mM when heated to 80 °C before use. This solution is stable for a few days but tends to precipitate when diluting it in a water-based buffer (Figure S27). Hence, the highest concentration that was possible to as-sess with our in vitro toxicity approaches was 100 µM of CU-DPPO. A direct comparison to existing literature on flame retardant toxicity is still possible in this concentration range.[48-50]

If the potential application of CU-DPPO requires higher doses, an alternative methodical approach should be considered. Collectively, our results show that CU-DPPO did not trigger an acute cytotoxic response in vitro in two model human cell lines (lung epithelial cells and macrophages).

Namely, no effect on cell viability was observed, ROS production, and cytokine release. CU-DPPO could be considered as a safe reactive flame retardant precursor, however, higher concentrations should be evaluated to have a better understanding of its activity. To assess the safety of CU-DPPO, an in vitro cytotoxicity platform was used.

Our screening approach monitored three indicators of toxicity: cell viability/death, ROS production and cytokine release. Cell death is a severe effect to treatment with CU-DPPO.

On the other hand, ROS and TNF-α production are measures of an acute sub-lethal cell stress.

In the present project, it has been determined that CU-DPPO did not cause measurable signs of toxicity up to a concentration of 100 µM. Even at 24 h of treatment, there was no effect on viability of lung epithelial cells and THP-1 macrophages. Our findings pro-vide new insights into the potential health impact of flame retardant precursors.

5   Conclusions

The synthesis of the bio-based additive was performed via phospha-Michael addition of diphenylphosphine oxide (DPPO) and coumarin. Initially, the feasibility of flame retardation of poly(lactic acid) (PLA) with a novel flame retardant (FR) additive was proved. The melt blended flame retardant-poly(lactic acid) PLA composites at 10 and 20 wt.% demonstrated improved fire performances achieving V-0 rating in the UL-94 test and obtaining an LOI value of 29 and 32 %, respectively. At the second step, the production of FR-PLA via a green reactive extrusion (REX) process proved to be possible in this work. The reactive extrusion (REX) technique process allowed for the preparation of flame retardant-poly(lactic acid) PLA in a one-pot two-step process, i) synthesis of PLA matrix ii) addition of CUDPPO FR, reducing a step in flame retardant composites manufacturing. The performance of the thermo-oxidative stability was proven via preliminary studies on molecular weight measurements of the R-PLA-CU-DPPO samples taken after the processing step. It was shown that the presence of 4-(diphenylphosphoryl)chroman-2-

one (CU-DPPO) in PLA matrix reduces the decrease of the molecular weight compared to blank neat REX-PLA (R-PLA). Thermal properties were studied by means of thermogravimetric analysis (TGA) showing a higher decomposition temperature for all the samples containing the CU-DPPO additive.

In line with the idea of bio-based materials, preliminary in vitro toxicity assessment indicated no adverse effects of the 4-(diphenylphosphoryl)chroman-2-one (CU-DPPO) on lung and immune cells lines. However, additional in vivo studies as well as eco-toxicological aspects have to be performed for a comprehensive assessment of potential health risks. Furthermore, health safety aspects of the final flame retardant product (not only of the new additive) have to be evaluated. This however is beyond the scope of the present study. Future work will also explore the use of other phosphorus-based additives using reactive extrusion (REX) technique process and optimizing the reaction conditions in order to increase the molecular weight of the composites.

Author contributions

D. Roncucci: Conceptualization, Methodology, Formal analysis, Investigation, Writing original draft, Writing - review & editing

M-O. Augé: Review & editing

S. Dul: Methodology, Formal analysis, Investigation, Writing - review & editing

J. Chen: Formal analysis, Investigation, review & editing

A. Gooneie: Investigation, review & editing.

D. Rentsch: Formal analysis, Writing - review & editing

S. Lehner: Formal analysis

M. Jovic: Formal analysis

A. Rippl: Investigation, Formal analysis, Writing - review & editing

V. Ayala: Investigation, Formal analysis, Writing - review & editing

F. Bonnet: Review & editing

S. Bourbigot: Review & editing

H. Grützmacher: Supervision, Writing - review & editing

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S. Gaan: Conceptualization, Funding acquisition, Supervision, Writing - review & editing

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.

Acknowledgments

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Flame Retardants: Prioritization in Search for Safe(r) Alternatives. *Arch Toxicol* **2014**, *88*,

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Lactide monomer

Bio-based CU-DPPO FR

Reactive extrusion (REX)

Flame retardant PLA composite
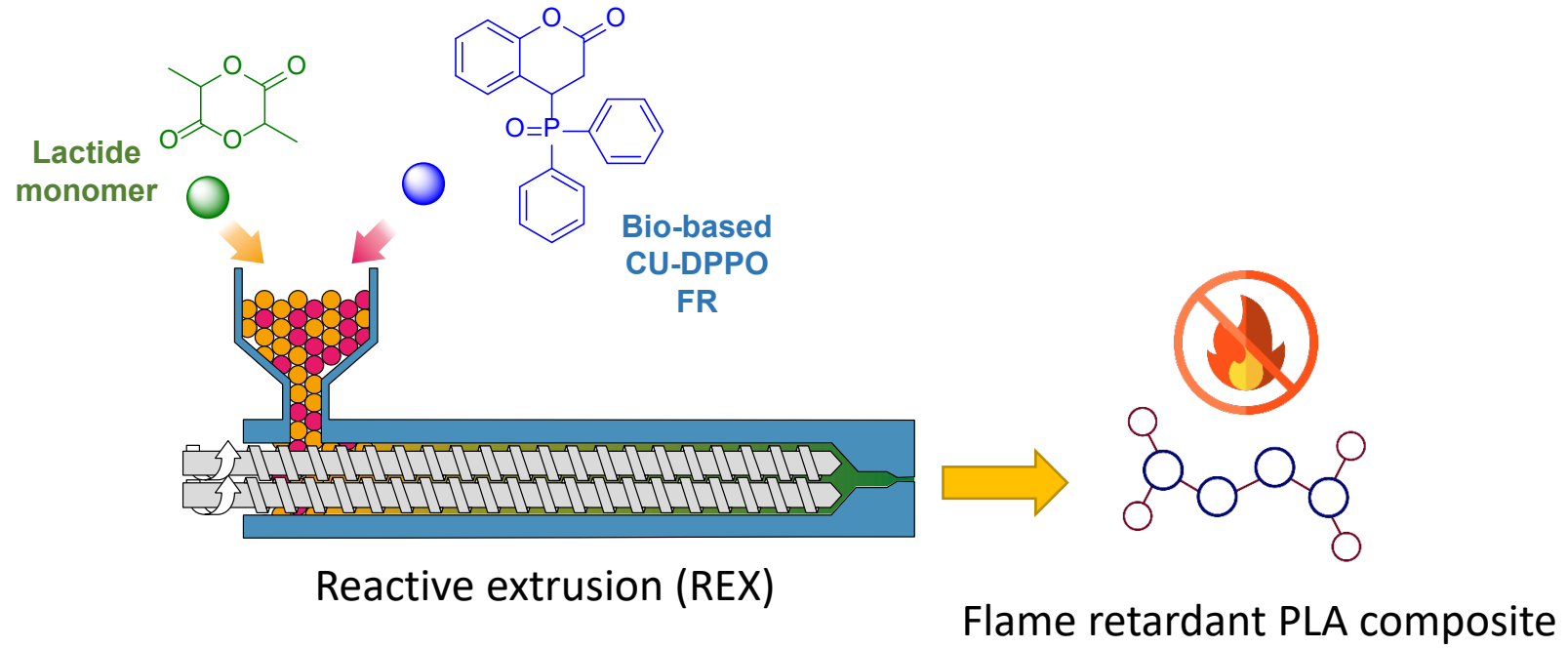
Supporting information

Coumarin-DPPO a new bio-based phosphorus additive

for poly(lactic acid):

Processing and flame retardant application

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5.1   NMR spectra
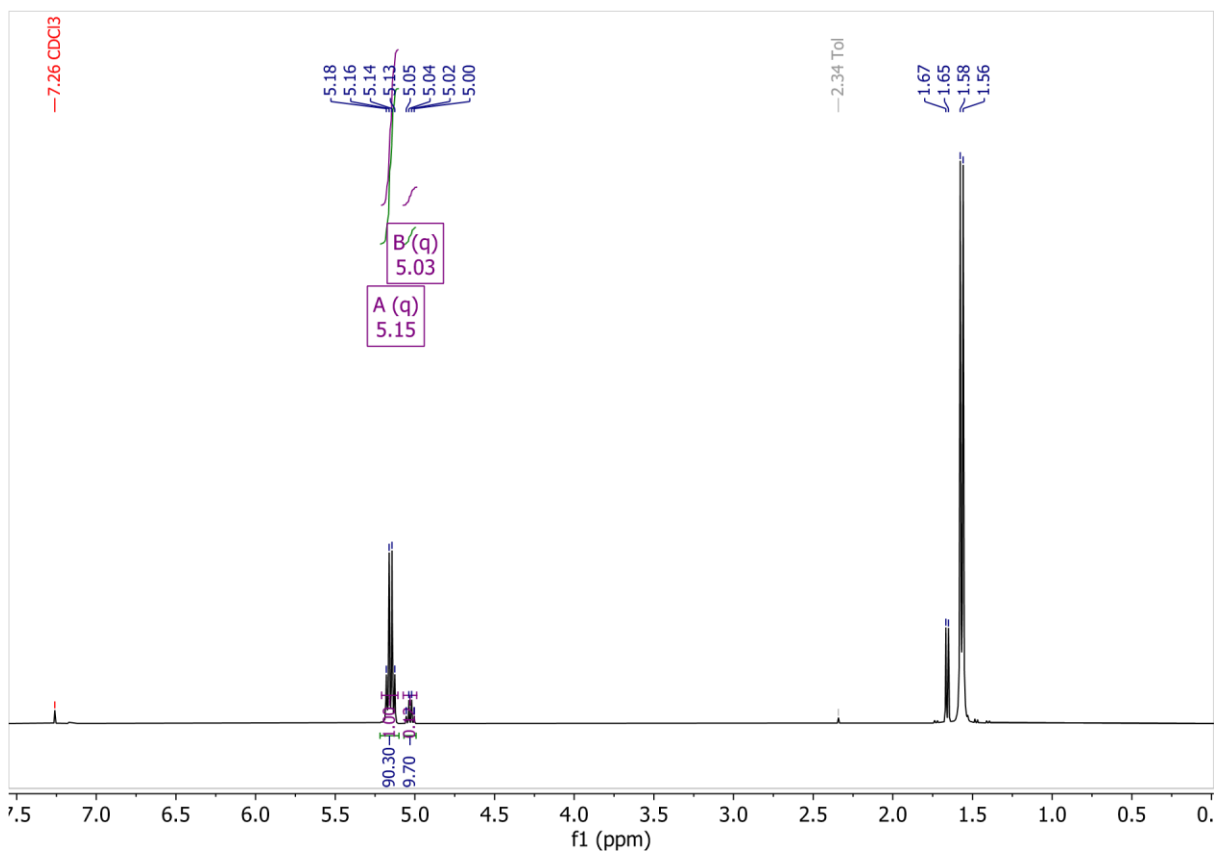
Figure S14: ^{1}H NMR spectrum of R-PLA (5.15 ppm), containing unreacted ($_L$)-lactide (5.03 ppm).
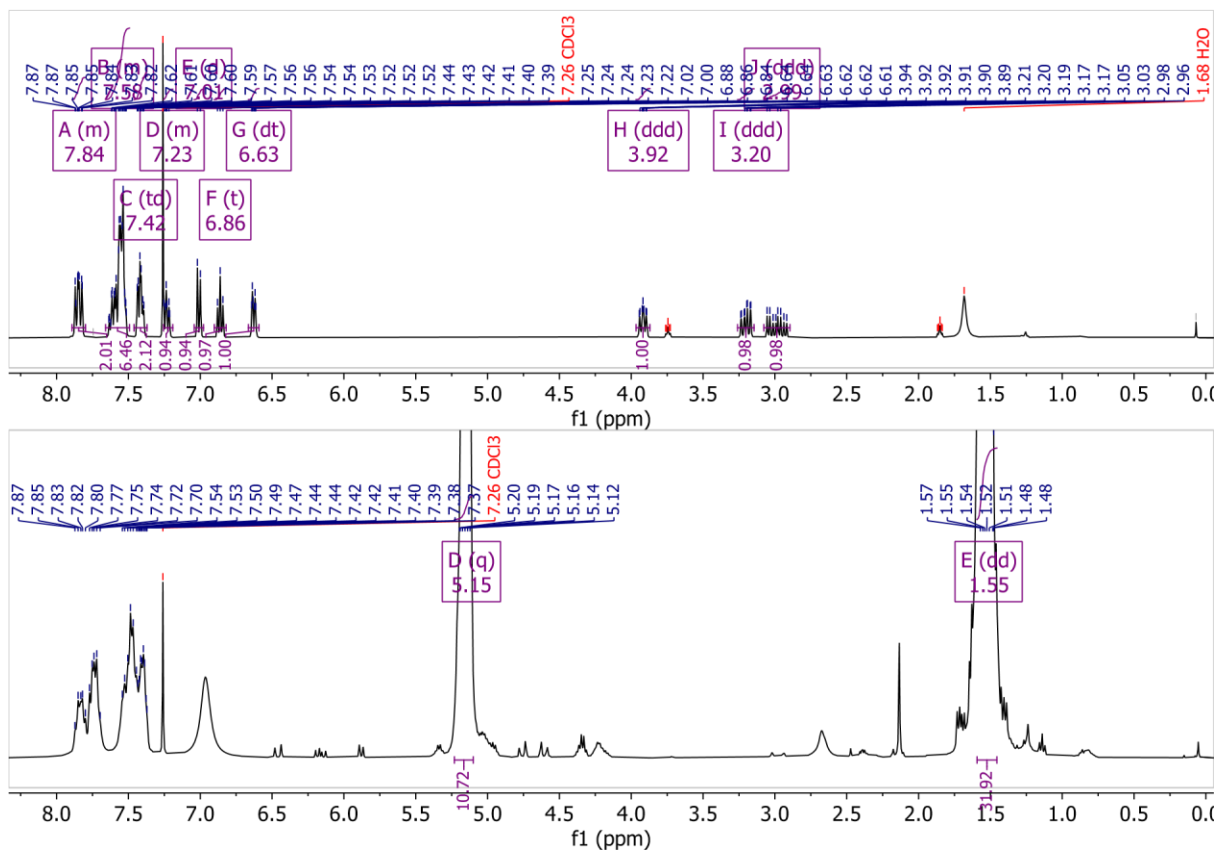
Figure S15: ^{1}H NMR spectrum of CU-DPPO monomer (top) and PLA/CU-DPPO thermally treated (bottom) showing decomposition products.
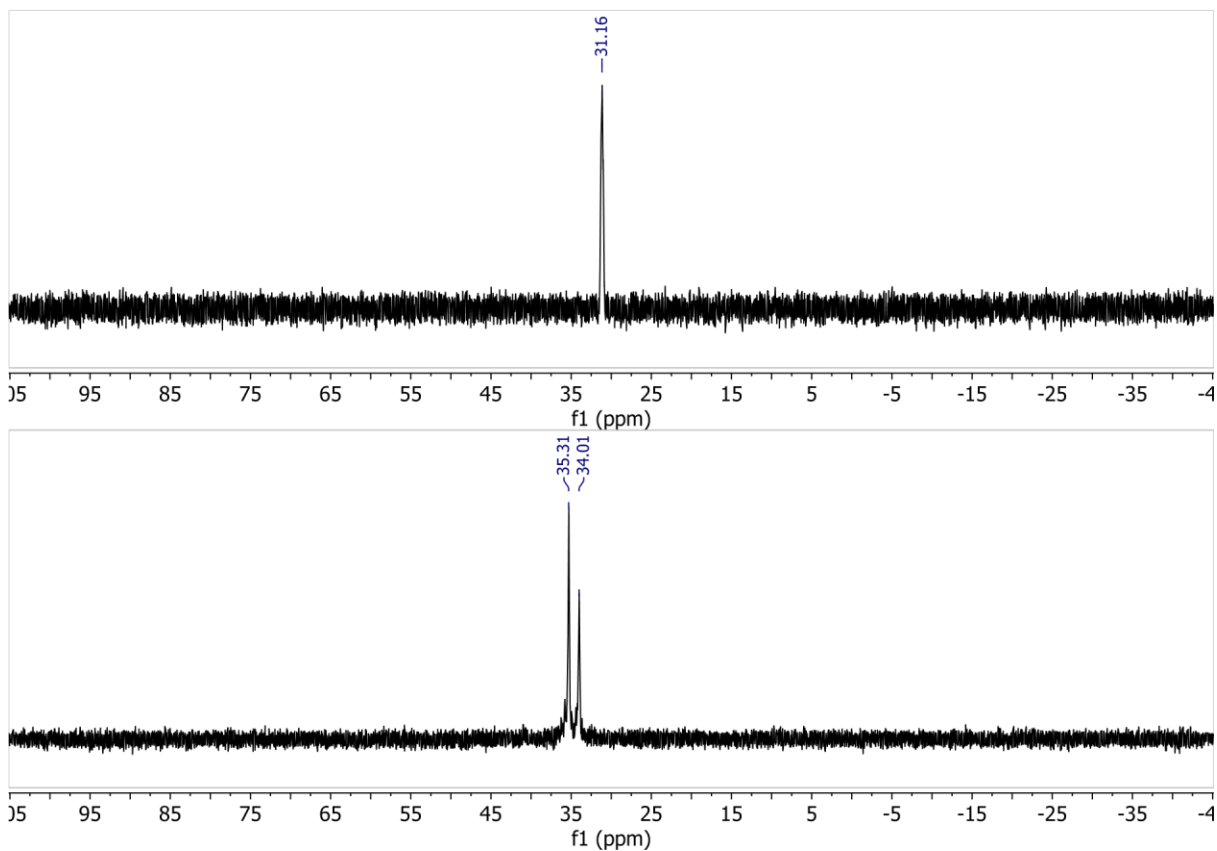
Figure S16: ^{31}P NMR spectra in d-chloroform of CU–DPPO monomer (top) and thermally treated PLA-CU-DPPO (bottom, heating up to 330°C) showing resonances of decomposition.
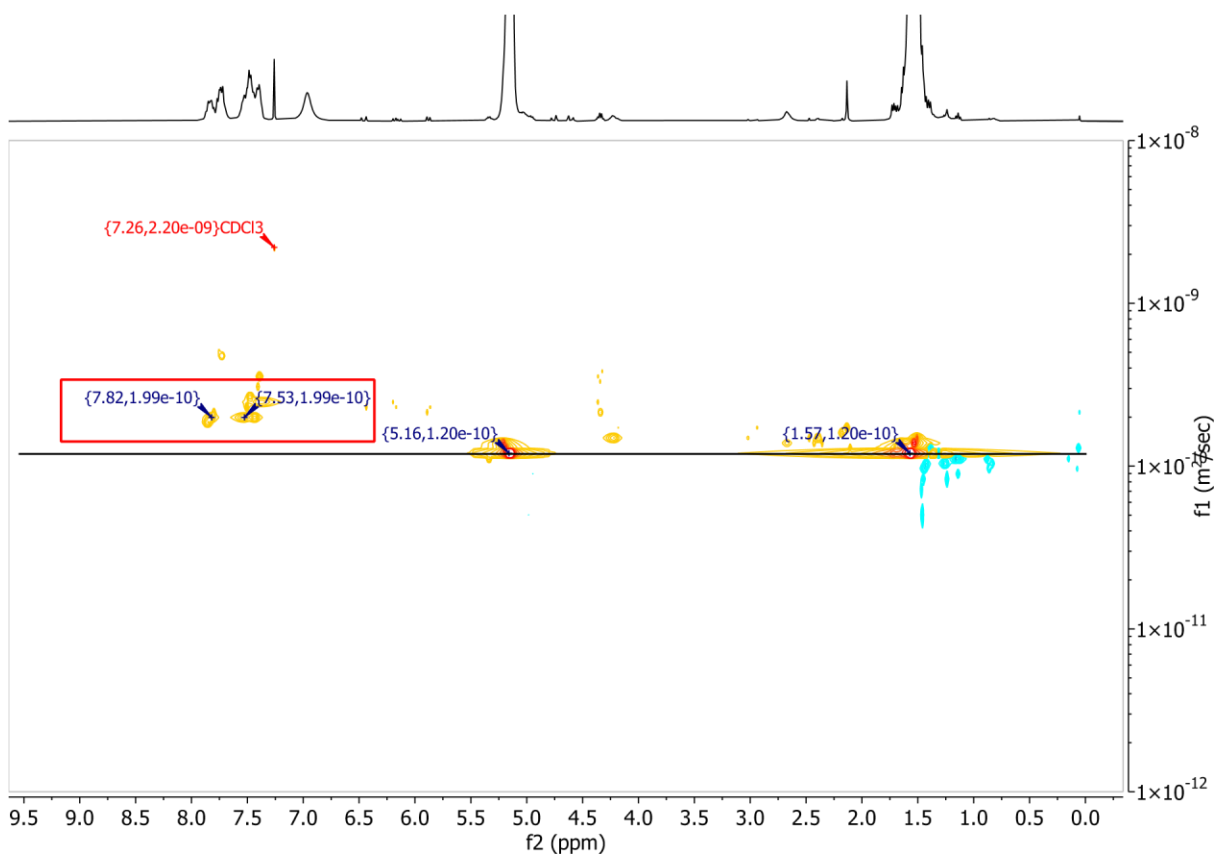
Figure S17: Diffusion-ordered spectroscopy (DOSY) NMR spectrum of PLA/CU-DPPO thermally treated at 330 °C. x axis: δ ^{1}H [ppm] y axis: D [m^2s^{-1}]. The first number in the curly brackets represents the chemical shift of the signal and the second number represents the diffusion coefficient. $D_{sol} = 2.20 \cdot 10^{-9} m^2 s^{-1}$, $D_{PLA} = 1.20 \cdot 10^{-10} m^2 s^{-1}$, $D_{CU-DPPO decomposition} = 2.00 \cdot 10^{-10} m^2 s^{-1}$.

5.2   Mass spectra
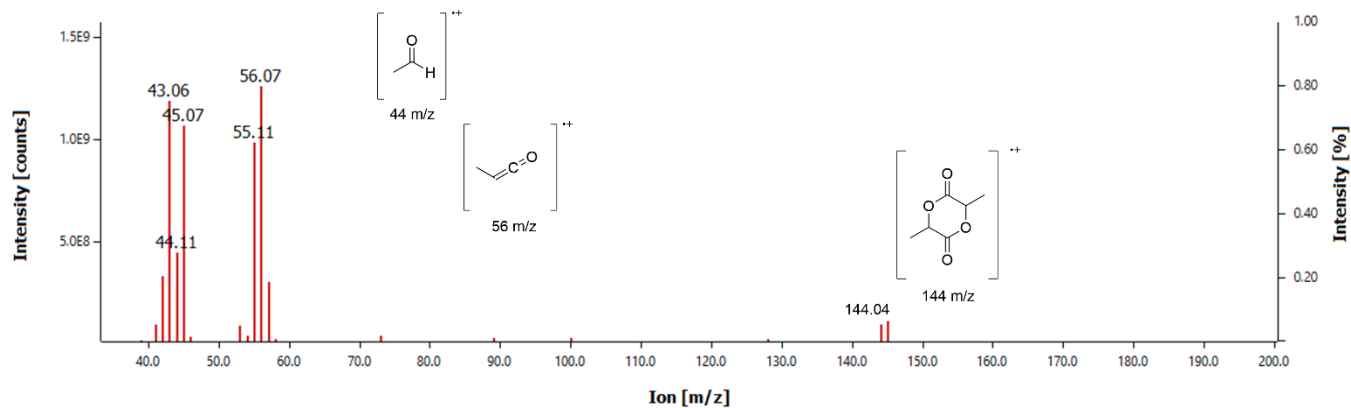
Figure S18: Mass spectrum of R-PLA at 3.6 min.
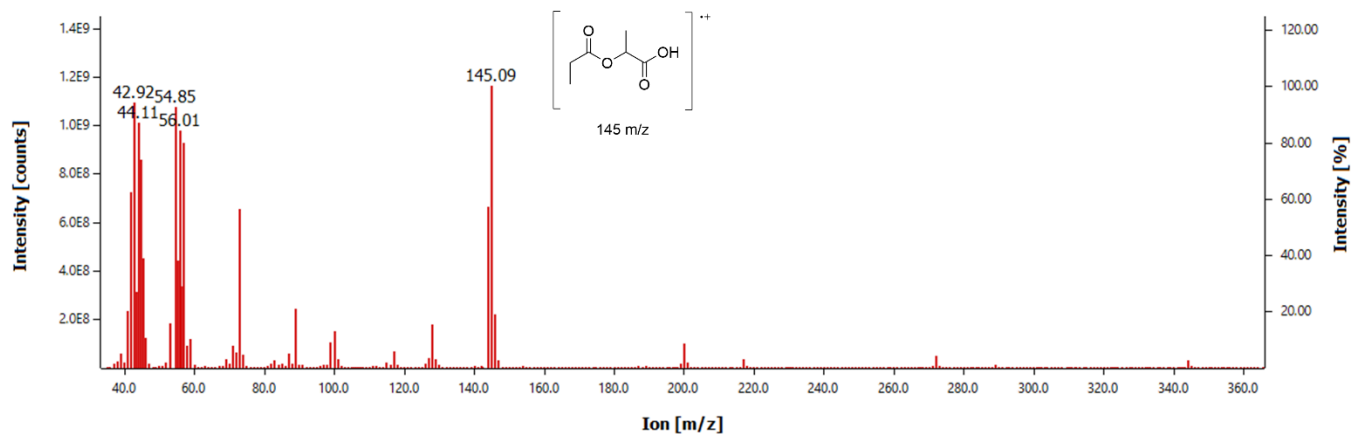
Figure S19: Mass spectrum of R-PLA at 6.8 min.
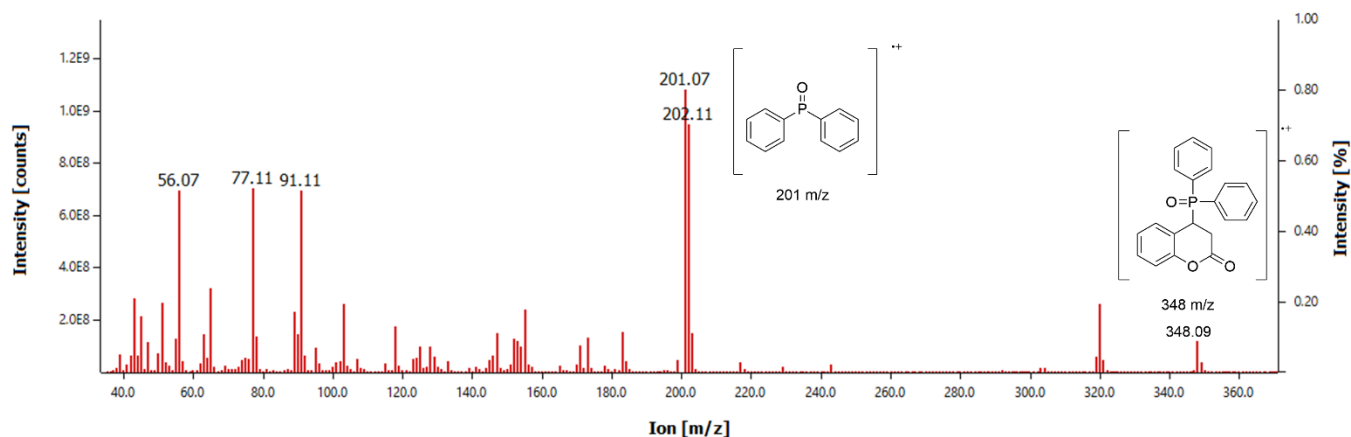
Figure S20: Mass spectrum of R-PLA-CU-DPPO at 6.5 min.
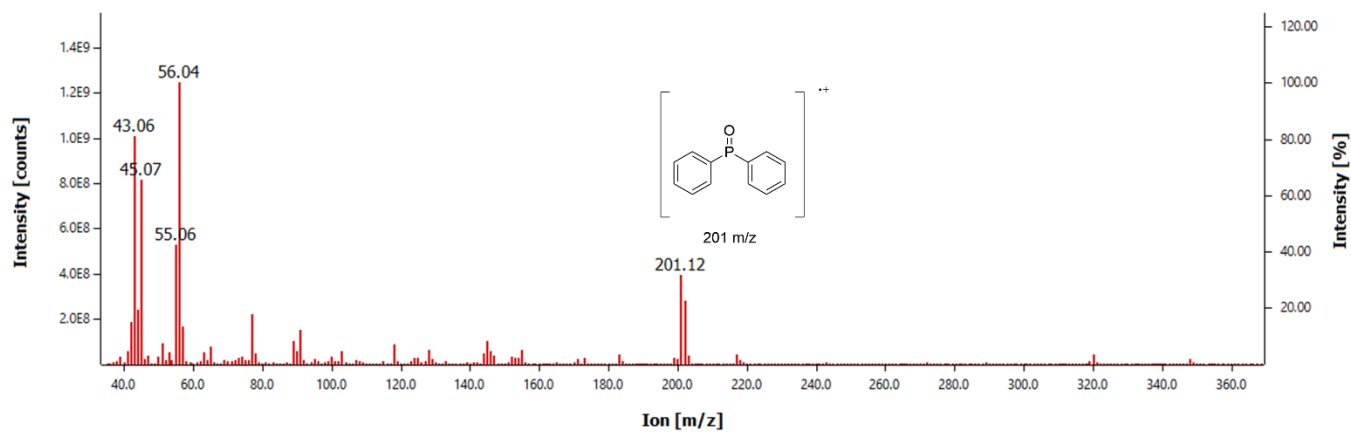
Figure S21: Mass spectrum of R-PLA-CU-DPPO at 7 min.
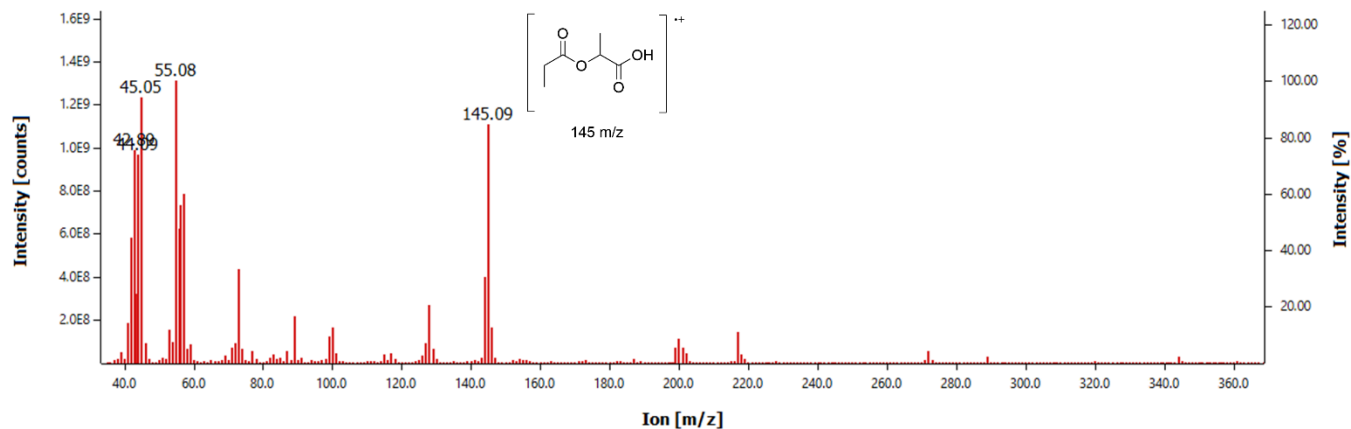
Figure S22: Mass spectrum of R-PLA-CU-DPPO at 7.7 min.
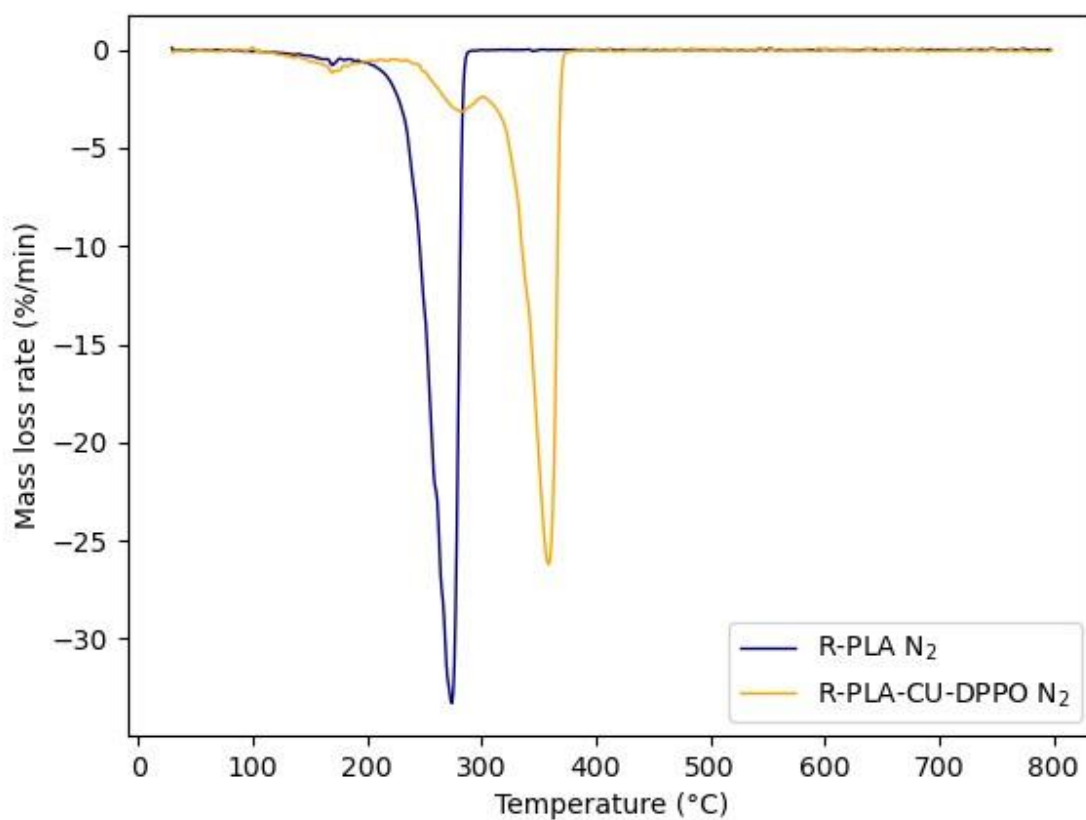
Figure S23: DTG of R-PLA and R-PLA-CU-DPPO.
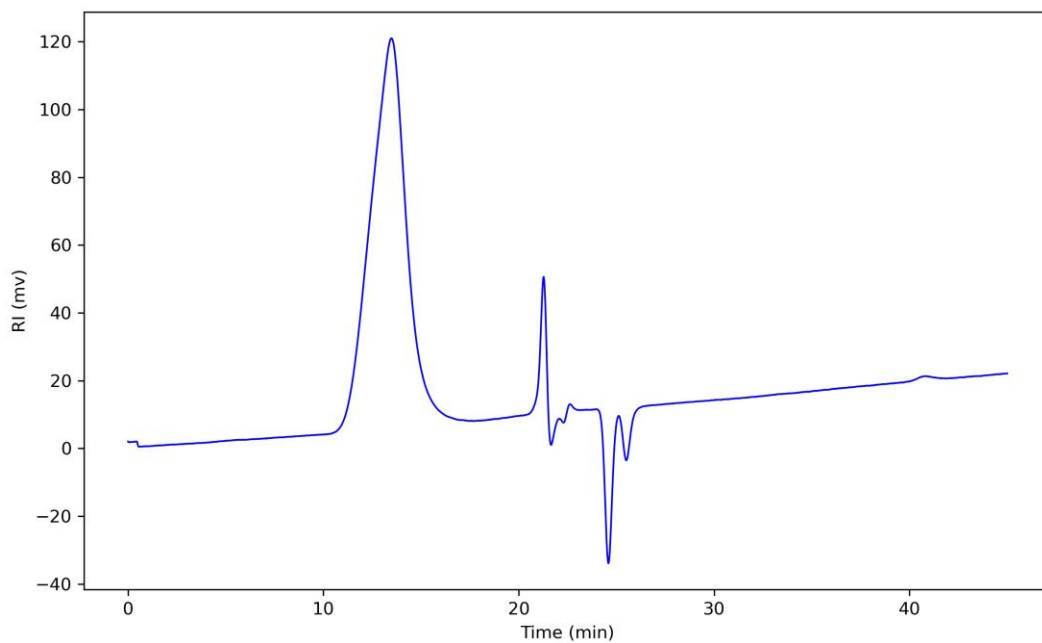
5.3   Selected SEC traces

Figure S24: Example SEC of R-PLA, Mn^{SEC} = 105.8 kgmol^{-1}, Đ = 1.6 (Entry 1, Table 4).
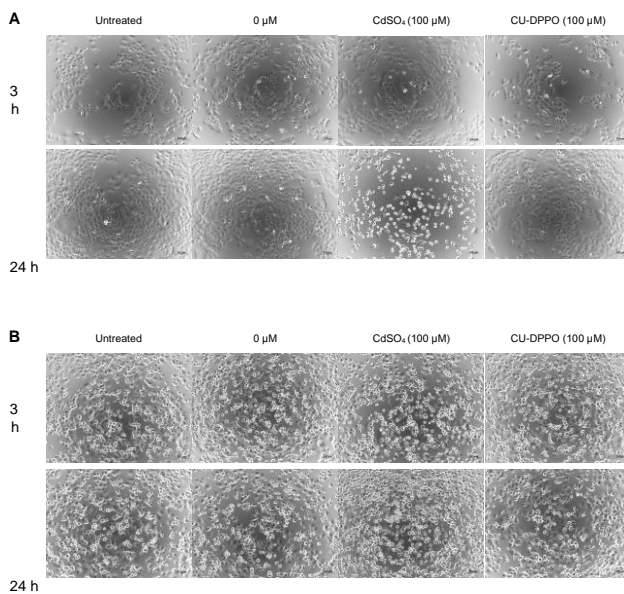
5.4   Toxicology
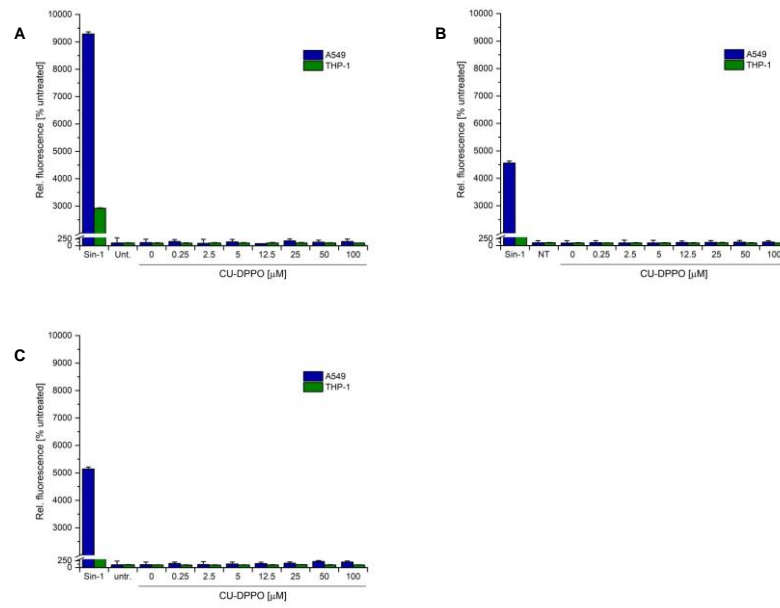
Figure S25: Cell viability.

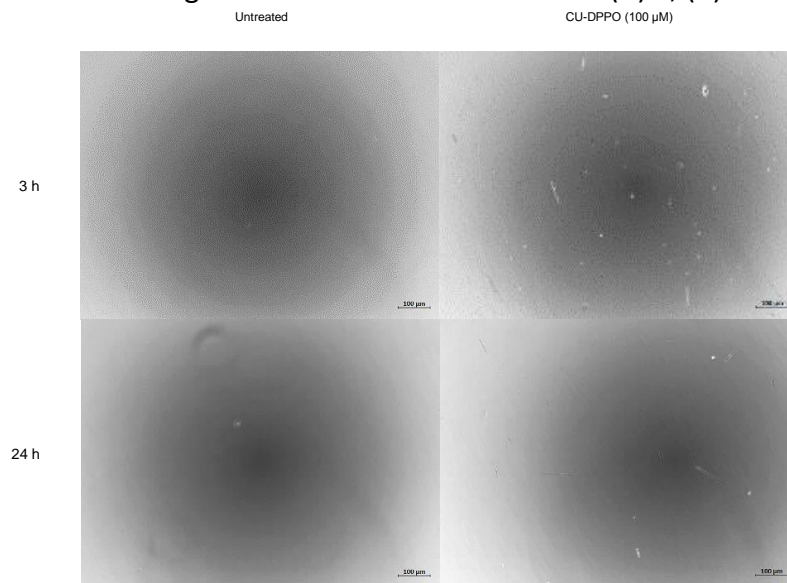Figure S26: ROS measurements at (A) 1, (B) 3 and (C) 4 h.

Figure S27: Sediments.

The authors declare no conflict of interest. We confirm that this submission is original; it is not published elsewhere and is not under consideration for publication elsewhere.