DNA as a Flame Retardant Additive for Low-Density Polyethylene

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Abstract

Deoxyribonucleic acid (DNA) was investigated as a flame retardant (FR) additive for melt-compounded formulations with low-density polyethylene (LDPE) and compared to LDPE compounded with melamine polyphosphate (MPP), one of the industry standard intumescent FR additives for plastics. DNA showed a much greater compatibility with the LDPE matrix than MPP. At high loading levels, DNA showed minimal increases in compounding torque, while MPP increased torque by over 20%. Qualitative evaluation using SEM and EDS showed that DNA/LDPE blends had significantly improved cross-sectional morphology, with fewer microaggregates and improved particle dispersion than MPP/LDPE. Horizontal burn testing showed that DNA markedly reduced flame burn distances in LDPE above loading levels of 5% w/w. Biochemical characterization of heat-treated DNA revealed that DNA undergoes denaturation, fragmentation, and

oxidation during compounding, but these processes did not appear to have an effect on its FR properties. This study expands the field of DNA-based FR beyond specialty substrates, and establishes DNA as a viable green FR additive for commodity polymer applications.

1. Introduction

Over the past 40 years, increasing global health concerns and regulatory scrutiny associated with organohalogen flame retardants (FR) has triggered a shift toward the development and use of non-halogenated FR agents.[1,2] Within the polymer industry, the most widely used non-halogenated FR additives are mineral fillers such as metal hydroxides, most commonly aluminum ($\text{Al}(\text{OH})_3$) and magnesium ($\text{Mg}(\text{OH})_2$).[3,4] Metal hydroxides are attractive FR additives owing to their endothermic degradation mechanisms and their ability to produce water vapor and metal oxide residue upon thermal decomposition.[5] Their low cost, natural abundance, and compatibility with a wide variety of polymer substrates have made inorganic hydroxides ubiquitous FR additives in many commodity plastics applications. However, the largest challenge facing the use of metal hydroxides is their significantly reduced FR performance relative to halogenated FR additives at comparable loading levels.[6] As a result, very high loading levels, up to 60% w/w, are often required to achieve desired FR performance.[6,7] At such high loading levels, the physical and mechanical properties of the polymer substrate are greatly compromised. As a result, additional plasticizing or

compatibilizing agents are often used in order to mitigate the deleterious effects of high mineral filler load, further driving up cost and complicating production processes.

In response to these challenges, non-halogenated FR additives derived from organic compounds are often used as alternatives or synergistic additives to mineral fillers.[5,8] One such class of FR additives is collectively known as intumescent flame retardants. These additives are known for their ability to promote the formation of thick, foamy char residue throughout the substrate upon ignition.[5,9] Among the most popular intumescent FR additives in industrial use are ammonium polyphosphate (APP) and melamine polyphosphate (MPP).[10–12] These compounds possess a unique FR mechanism that allows them to deliver potent FR properties to polymers at significantly lower loading levels than most mineral fillers. The intumescent properties of both APP and MPP are marked by the presence of an acid source such as phosphate, an additional carbon source such as pentaerythritol, and nitrogenous blowing agent such as ammonium or melamine. Upon thermal decomposition, the phosphate groups act as acid precursors, catalyzing carbonization throughout the polymer substrate and promoting char layer formation at the substrate-flame interface.[5,9] Meanwhile, the nitrogen-containing moieties decompose to inert nitrogenous gasses such as nitrogen or ammonia. When occurring simultaneously, the nitrogenous gas promotes the formation of a thick foamy (intumescent) char residue, thus increasing the distance between the polymer substrate and the flame.[5] In addition, the nitrogen gas also acts to dissolve oxygen that is present at the substrate-flame interface, further reducing flame development, and can promote self-extinguishment. Finally, studies have shown

that effective char formers such as intumescent FR agents also tend to reduce smoke production, as many volatile products of degradation are retained more readily in the char.[13]

The use of deoxyribonucleic acid (DNA) was recently described as a coating for textile and polyurethane foam applications.[14–18] Much like APP and MPP, DNA is rich in both phosphate and nitrogen. Adjacent nucleic acids in DNA are attached through phosphodiester linkages, while the nucleobases are nitrogen rich (**Figure 1**). In addition, Malucelli and coworkers concluded that the deoxyribose units in DNA also act as a carbon source, allowing DNA to act as “all-in-one” intumescent FR additive.[14,15] A coating of DNA was shown to drastically reduce ignitability of cotton fabrics when exposed to both direct flame and irradiative heat.[14] Additional studies also investigated DNA as an additive for melt-blended formulations with ethylene-co-vinyl acetate (EVA) and concluded that DNA imparts similar FR properties to the EVA substrate as APP.[19,20] The primary disadvantage of using DNA as an FR additive in large scale is its high cost, mostly associated with the high purity required for applications in molecular biology.[21] However, recent advances in DNA extraction from large-scale sources such as salmon milt and agricultural waste, together with coarse extraction techniques such as thermal or alkaline lysis of bacterial cultures have great potential to drive costs down. Also, since DNA behaves as an all-inclusive intumescent FR agent, DNA-based FR formulations may represent additional cost reductions due to the potentially reduced need of carbon source additives. Since FR applications would

not need rigorous purification, it becomes possible for DNA prices to become competitive with popular intumescent FR products.[22–24]

Figure 1. Chemical structures of deoxyribonucleic acid (DNA, single strand, guanine base shown for clarity) and melamine polyphosphate (MPP).

In order to further expand the field of DNA-based FR applications beyond coatings, textiles, and specialty substrates, this study serves to investigate its FR properties as a melt-compounded additive with low-density polyethylene (LDPE). LDPE constitutes one of the largest single market segments of commodity thermoplastics worldwide owing to its low cost of production and excellent processing performance at relatively low temperatures, typically around 110 °C.[25] However, its extremely poor native FR properties and bulk flammability have necessitated the use of FR additives in many commercial applications.[26–28] Together, LDPE’s exceptionally high commercial popularity, low melt-processing threshold, and poor FR performance make it an excellent model substrate for potential new FR additives. Here, the FR performance of DNA is compared to that of melamine polyphosphate (MPP). Over the past 40 years MPP has become ubiquitous as a powerful intumescent FR additive and synergistic co-

additive for plastics.[29] However, recent studies have generated increased concern over the biological toxicity of melamine, a major product of decomposition of MPP.[30] These findings further create impetus for the development of biologically safe FR additives with a wider range of commercial applications.

In this study, the thermal decomposition, mechanical properties, and FR performance of DNA/LDPE blends were evaluated at loading levels between 5% and 40% w/w. Overall, DNA demonstrated greatly improved compatibility with LDPE than MPP. At high loading levels, DNA minimally increased compounding torques while MPP increased torque by 20%. SEM revealed that DNA dispersed much more homogeneously throughout the LDPE substrate, with reduced particle aggregation over MPP. Tensile testing revealed that DNA/LDPE compounds had generally improved mechanical strength over MPP/LDPE, with increased modulus and elongation at higher loading levels. Finally, DNA/LDPE compounds showed markedly reduced burn distance in the horizontal configuration than MPP/LDPE above a loading level of 5% w/w. In order to further understand the thermal behavior of DNA during processing, biochemical characterization of both neat and heat-treated DNA was conducted. DNA was shown to undergo denaturation, fragmentation, and oxidative processes that include the production of 8-oxo-guanine species at temperatures of 145 °C.

This study has established DNA as a potent flame retardant additive for applications in melt-compounded low-density polyethylene (LDPE). LDPE formulations with DNA resulted in comparable reductions in heat release and breaking strain when compared

to those with melamine polyphosphate (MPP), one of the industry standard intumescent FR additives for plastics. However, DNA showed significantly improved compatibility with the LDPE matrix, drastically reduced burn distances in the horizontal configuration, early-onset solid char formation, and enhanced tensile modulus at higher loading levels.

2. Experimental

2.1 Materials

Deoxyribonucleic acid powder from herring sperm (<50 bp degraded), α -cyano-4-hydroxycinnamic acid (Sigma-Aldrich), melamine polyphosphate (Hangzhou JLS Flame Retardants), DOW low-density polyethylene 503A (MFI 2, DOW Chemical), potassium phosphate monobasic anhydrous, sodium phosphate dibasic anhydrous, sodium chloride, agarose I, ethidium bromide, acetonitrile, trifluoroacetic acid, exACTGene 100 bp DNA ladder (Fisher Scientific, St. Louis, MO). All reagents were used directly, without further purification.

2.2 Thermal Analysis

Thermogravimetric analysis (TGA) was performed under both nitrogen and air atmospheres using a TA Instruments Q500 TGA instrument, equipped with a platinum pan. Samples were subjected to a 10-minute drying step at 100 °C prior to heating at 1, 10, or 20 °C/min to either 700 or 900 °C. Differential scanning calorimetry (DSC) was

performed using a TA Instruments Q100 DSC instrument equipped with T-Zero hermetic aluminum pans. Heating cycles were typically in the range of -50 – 200 °C with a heating rate of 10 °C/min. All starting materials were analyzed in sealed pan configuration, and DNA was also analyzed in open pan configuration, equilibrated to either air or nitrogen atmospheres.

2.3 Characterization of Heat-Treated DNA

DNA heat treatment was performed using a TA Instruments Q100 differential scanning calorimeter (DSC) equipped with T-Zero hermetic aluminum pans. DNA samples were equilibrated to -50 °C, then heated to 145 °C at a rate of 10 °C/min and held at 145 °C for 5 min. The heat-treated DNA was then either analyzed as a solid or dissolved in phosphate buffered saline (pH 7.4). Fast protein liquid chromatography (FPLC) analysis was performed using a GE Healthcare AKTA-FPLC 900 chromatography system equipped with a GE Healthcare Superdex 75 10/300 GL column. The mobile phase was 100 mM phosphate buffered saline, with 150 mM NaCl (pH 7.4) at a flowrate of 0.4 mL/min. Samples of neat or heat treated DNA were analyzed at a 5 mg/mL concentration with an injection volume of 100 µL. UV-vis spectra were collected using a Shimadzu Biospec-Nano spectrophotometer with a pathlength of 0.7 mm and a concentration of 0.5 mg/mL. Agarose gel electrophoresis was performed with a 2 wt% agarose gel with 0.001 v/v of 10 mg/mL ethidium bromide. Samples were run at 5 mg/mL (as determined via absorbance), 140V, for 60 minutes and transilluminated with UV light. The results were compared against a 1000 – 100 base pair ladder. Matrix-

assisted laser desorption-ionization/time-of-flight mass spectroscopy (MALDI-TOF MS) was performed using a Bruker Autoflex III MALDI-TOF-TOF mass spectrometer equipped with a 200 Hz Smartbeam II laser system. Samples were desalted using a C18 ZipTip column and deposited with a saturated CHCA matrix onto a Bruker ground steel target plate accessory.

2.4 Compounding and Extrusion

LDPE was compounded and extruded with deoxyribonucleic acid (DNA) or melamine polyphosphate (MPP) using a Thermo Haake Minilab twin-screw microcompounder at loading levels of 5, 10, 20, 30, or 40 w/w. All samples were compounded at a screw speed of 80 rpm for 2 minutes at temperatures of 145 °C for samples containing DNA and 165 °C for samples containing MPP, and extruded through a 2 x 5 mm (0.1 cm²) rectangular die. Samples cooled at room temperature for 24 hours prior to analysis.

2.5 Pyrolysis

Microcombustion calorimetry (MCC) analysis was carried out on an FAA Microscale Combustion Calorimeter. For all experiments, the heating cell was equilibrated to 50 °C and a nitrogen/oxygen ratio of 20%/80%, and the pyrolyzer cell equilibrated to 900 °C. Samples of approximately 4.0 mg were subjected to a heating rate of 60 °C/min to a temperature of 700 °C. For each loading level, samples were run in triplicate and heat release rate (HRR) and total heat release (THR) profiles are reported as averages.

2.6 Fourier-Transform Infrared (FT-IR) Spectroscopy

Spectra were collected using a SpecAgilent Cary 630 FT-IR spectrometer in ATR mode in the range of $4000 - 600 \text{ cm}^{-1}$ with a total of 4 scans at an 8 cm^{-1} resolution. For FT-IR experiments, neat samples were in powder form and compounded samples were cut lengthwise to collect spectra from the internal surface.

2.7 Scanning Electron Microscopy (SEM)

SEM/EDS analysis was performed on a Nova NanoLab 200 FEG-SEM/FIB microscope combined with an XMax 50 mm² EDS system. Compounded and extruded samples were cryo-microtomed at $-13 \text{ }^{\circ}\text{C}$ with a glass knife, mounted with carbon tape, and sputtercoated with a 5 nm thick layer of palladium. Elemental spectra were collected at 10 kV for 15 minutes.

2.8 Tensile Testing

All tensile testing was conducted using an *MTS 2525 ReNew 5565* electromechanical extensometer equipped with a 1 kN load cell, with a sample length of 10 cm, initial gauge length of 2.5 cm at a strain rate of 20 mm/min. Tensile modulus and elongation at break measurements were reported as averages of five replicate runs at each loading level.

2.9 UL-94 Horizontal Burn Testing

Horizontal burn testing was performed based on a modified version of the ASTM D635-14 protocol.[31] Samples of 13.0 cm in length were mounted on a 1 cm wire mesh and exposed to direct flame for 10 s. Measurements of flame spread rate and burn distance measurements were reported as averages of 4 replicate samples at each loading level.

3. Results and Discussion

In order to establish the optimum melt-compounding conditions for deoxyribonucleic acid (DNA), thorough thermal characterization was performed using thermogravimetry (TGA) and differential scanning calorimetry (DSC). TGA analysis at 10 °C/min under nitrogen atmosphere demonstrated that DNA has an onset of thermal decomposition at around 170 °C, corresponding to a minor weight loss of 2%, and a second more pronounced weight loss event at ~220 °C (**Figure 2A,B**). These weight loss events have been previously described in TGA studies of solid state DNA.[15] In order to further confirm the first weight loss, and distinguish it from the potential loss of any strongly bound water, TGA was also performed at 1 °C/min (high-resolution TGA) and at 20 °C/min. The weight loss onset shifted slightly in response to changes in the heating rate and the overall weight loss percentage remained the same (**Figure 2D,E**). These findings are in good agreement with previous studies by Malucelli et al, concluding that this first weight loss corresponds to the evolution of isocyanic acid.[15] Beyond 200 °C, DNA undergoes gradual decomposition over a very wide temperature range. TGA of

DNA under air atmosphere showed similar weight loss behavior in this range (**Figure S1**). DSC analysis of DNA in the solid state showed a broad endotherm at 110 °C, probably corresponding to melting of the solid material. Immediately following the endotherm, a sharp exothermic peak is also seen at approximately 140 °C (**Figure 2C**).

This coupled endothermic/exothermic behavior is likely attributed to the denaturation of the DNA immediately following melting, and subsequent reorganization of the strands or fragments into a lower-energy state. This behavior is consistent with a previous TGA study that concluded that the gradual weight loss of DNA beyond 180 °C is attributed to reorganization events that stabilize the DNA throughout heating.[15] This behavior is not observed in DSC studies of DNA in solution, as the DNA is able to denature at much lower temperatures (typically between 50-90 °C), and more readily assume low-energy conformations owing to the larger possible number of degrees of freedom.[32] The coupled endothermic/exothermic behavior is no longer observed in subsequent heating cycles, likely as a result of additional degradative processes that prevent re-annealing, however, a glass transition remains at 110 °C, as observed during the first heating cycle (**Figure 2F**). The melt-endotherm and onset of thermal decomposition points for the low-density polyethylene (LDPE) used in this study were also verified using TGA and DSC (**Figure 2A, 2B, S1**). LDPE was found to have a sharp melt-endotherm peak at 110 °C (**Figure S2**) and an onset of thermal decomposition of ~430 °C, as measured under nitrogen atmosphere (**Figure 2A,B**). These values were in good agreement with those for most LDPE products reported in literature.[33,34] Given its low onset of thermal decomposition, DNA is a particularly good candidate for applications in low-melt

polymer systems. In the case of the LDPE used in this study, high-temperature applications are typically not possible by virtue of its low melting temperature and therefore the onset of thermal decomposition of DNA at $\sim 170^\circ\text{C}$ is not strictly limiting. In fact, many commercial varieties of LDPE possess bulk melting temperatures below 170°C . In addition, the thermal decomposition of DNA involves the formation of nitrogenous gasses even at lower temperatures and is therefore still beneficial to flame retardance.[15]

Figure 2. Thermal characterization of LDPE, DNA, and MPP starting materials. (A) TGA thermograms and (B) derivative TGA of starting materials under nitrogen atmosphere. (C) DSC profile of DNA during the first heating cycle. (D) TGA thermograms and (E) derivative TGA of the first onset of decomposition at 170 °C of DNA at 1, 10 and 20 °C/min. (F) DSC profile of DNA during second, and subsequent heating cycles.

Based on these initial thermal studies, the compounding temperature for DNA and LDPE was selected to be 145 °C, as the lowest possible temperature at which DNA was likely to be sufficiently melted to properly incorporate into the LDPE substrate. The compounding temperature for MPP and LDPE was selected to be 165 °C, as is typical for LDPE.[35] Attempts at compounding MPP with LDPE at 145 °C were not successful due to extremely high resultant shear torques and poor consistency of the compounded material. In both cases, a screw speed of 80 rpm was used. At this speed, compounding residence time was kept below 2 minutes, while ensuring adequate dispersion of the additive. The additives were considered to have achieved maximum dispersion when the average compounding torque measurement had stabilized and did not deviate beyond ± 3.0 N·cm. Average stabilized compounding torques were recorded for both additives at each loading level. Compounding torques of neat LDPE at equal batch weight was also recorded at both 145 °C and 165 °C, as a negative control for samples containing DNA and MPP, respectively. The torque measurement for neat LDPE at 165 °C was 51 N·cm, and increased markedly with increased MPP loading levels, reaching a maximum of 73.8 N·cm at an MPP loading level of 40%. On the other hand, at 145 °C, the torque of neat LDPE was 58.7 N·cm, but increased only gradually with

increased DNA loading level, reaching a maximum of only 63.4 N·cm at a DNA loading level of 40% (**Figure 3**). Overall, the compounding torque of DNA/LDPE at 145 °C was lower than that of MPP/LDPE at 165 °C at loading levels greater than 20%. These measurements indicate a greater degree of compatibility between the substrate and the DNA.

Figure 3. Average compounding torque during processing of DNA/LDPE and MPP/LDPE formulations. All DNA compounding was performed at 145 °C, all MPP compounding was performed at 165 °C.

In order to further examine the compatibility between DNA and LDPE following compounding, cross-sectional morphology and particle dispersion were evaluated via scanning electron microscopy (SEM) coupled with energy-dispersive spectroscopy (EDS). SEM revealed that DNA dispersed much more homogeneously into the LDPE substrate than MPP. Qualitative evaluation of SEM micrographs showed that compounded DNA/LDPE had significantly decreased microaggregation, cratering, and bubbling throughout the material relative to MPP/LDPE (**Figure 4**). This difference is

indicative of a greater compatibility or miscibility between the LDPE and DNA. These findings are in agreement with previous studies investigating the bulk compatibility between DNA and ethylene vinyl acetate (EVA), where DNA was also shown to incorporate homogeneously with the polymer substrate.[19,20] Since both DNA and MPP are rich in phosphorus, following the phosphorus signal via EDS provided an effective way to study particle dispersion. EDS revealed that, in spite of lower processing temperatures and shorter processing times, DNA disperses more homogeneously throughout the LDPE substrate than MPP (**Figure 4, S3**).

Figure 4. SEM micrographs (top) and EDS spectra (bottom) of compounded materials.

A. LDPE; **B.** DNA/LDPE and **C.** MPP/LDPE collected at 50 µm resolution.

Sample	TGA N ₂			TGA Air			MCC			
	T _{o,1} (°C)	T _{o,2} (°C)	Residue (700°C, %)	T _{o,1} (°C)	T _{o,2} (°C)	Residue (700°C, %)	PHRR (°C)	PHRR (W/g)	THR (W/g)	Residue (700°C, %)
LDPE	432.9	-	0	336.5	-	0	504.7	1123.4	46.8	0
DNA	172	212.1	41.7	169.8	208.1	41.7	-	-	-	-
MPP	346.2	-	35	337.2	-	31	-	-	-	-
DNA 5%	196.3	428.5	2.1	209.9	386	1.3	502.3	1121.2	44.8	2
MPP 5%	352.1	430.3	1.5	320.8	-	2	500.7	1057.7	46.4	1.5
DNA 10%	204.5	428.7	4.5	205.1	388.2	3.7	500.1	1045.7	43	4.6
MPP 10%	346.6	429.4	3.8	359.9	-	2.4	501.1	1007.3	42.7	3.9
DNA 20%	204.2	427.4	8.6	210.5	438.5	5.5	501.4	986.2	41	7.4
MPP 20%	347.7	427.6	6.4	367.1	-	4.1	495.2	849.5	38	6.9
DNA 30%	200	429.3	13.8	195.5	399	8	497.8	863.3	39.2	10.6
MPP 30%	351.3	431.4	9.1	357.6	-	8.9	500.5	750.1	36.9	11
DNA 40%	199.4	431	16.7	195.5	382.9	11.4	499.7	695.5	31.3	17.8
MPP 40%	353.6	431.3	11.9	367.7	-	12.1	501.3	701.6	33.4	12.7

Table 1. Thermal characterization of LDPE, DNA, MPP, and compounded materials.

Thermal analysis of compounded materials was performed at all loading levels (**Table 1**). TGA under nitrogen atmosphere showed two significant weight loss events for both DNA/LDPE and MPP/LDPE. The first weight loss event (T_{o,1}) occurred at ~200 °C for all samples containing DNA and at ~350 °C for all samples containing MPP. This weight loss corresponds to the initial degradation of the additive. The second weight loss (T_{o,2}) occurs at 430 °C in all cases, and corresponds to the decomposition of LDPE. By the time the substrate degrades above 430 °C, DNA has undergone a weight loss of almost 40% (**Figure 5A**). This is in contrast to only 20% in the case of MPP (**Figure 5B**). Although this early onset and greater percent decomposition of DNA limits its use in high-temperature processing applications, its more gradual decomposition over a wider temperature range versus MPP may be beneficial to its FR properties (**Figure 5C,D**). By allowing phosphorus-mediated carbonization as well as inert nitrogenous gas evolution to initiate at a lower temperature, DNA can more readily act as an effective char former

upon ignition. TGA of compounded materials under air atmosphere showed similar degradation profiles, however, the tendency of the material to ignite at high temperatures in the presence of oxygen contributed to interference that prohibited accurate identification of onset temperatures and residue yields (**Figure S4, Table 1**).

Additionally, TGA under nitrogen atmosphere was also used to measure the residue yield of DNA/LDPE and MPP/LDPE compounded samples at 700 °C. The residue yields at this temperature were compared to that of neat DNA and MPP additives in order to determine potential char production and decomposition delay effects given by the additives. Under nitrogen atmosphere neat LDPE undergoes rapid, abrupt, and complete thermal decomposition at ~450 °C (**Figure 2A**). Barring all other potential processes, the residue yield of compounded samples beyond ~450 °C is expected to only be from that of the additive. On this basis, any increases in residue yield above what is expected for the respective additive is likely indicative of either char formation as a result of carbonization of the substrate or delayed substrate degradation. The residue yields of DNA/LDPE samples were larger than expected for neat DNA at all loading levels whereas those of MPP/LDPE were lower than expected for all loading levels except 10% (**Table 1**). These findings indicate that DNA either participates in early-onset char formation throughout the LDPE or altogether postpones its thermal degradation.

Figure 5. TGA characterization of compounded materials. (A) TGA thermograms and (B) derivative TGA of DNA/LDPE compounds under nitrogen atmosphere. (C) TGA thermograms and (D) derivative TGA of MPP/LDPE compounds under nitrogen atmosphere. Solid: DNA or MPP, dashed: LDPE, red: 5%, orange: 10%, green: 20%, blue: 30%, purple: 40%.

Heat release profiles via microcombustion calorimetry (MCC) showed that neither DNA nor MPP cause any significant shift in the peak heat release time (PHR) at any loading levels studied (**Figure 6A,B**). Peak heat release rates were generally centered around ~500 °C. The total heat release (THR) profiles for DNA/LDPE and MPP/LDPE showed significant but similar reductions in net THR with increased additive loading levels (**Figure 6C,D**). In both cases, THR reached a minimum of approximately 700 W/g at

loading levels of 40%, representing an overall 40% reduction in THR over neat LDPE at 1123 W/g. The decrease in net THR trended linearly with increased loading levels of both DNA and MPP. Overall, the residue yield following pyrolysis of DNA/LDPE compounds was generally higher than that of MPP/LDPE (**Table 1**).

Figure 6. MCC profiles of compounded materials. (A) Heat release rate (HRR) and (B) total heat release (THR) profiles of DNA/LDPE measured at 1 °C/sec. (A) Heat release rate (HRR) and (B) total heat release (THR) profiles of MPP/LDPE measured at 1 °C/sec. Dashed: LDPE, red: 5%, orange: 10%, green: 20%, blue: 30%, purple: 40%.

Tensile strength testing was conducted on compounded samples at all loading levels at a rate of 20 mm/min. DNA/LDPE and MPP/LDPE had similar gradually-increasing trends in elastic modulus at loading levels of up to 20% (**Figure 7A**). At 30% and 40% DNA/LDPE showed a pronounced increase in elastic modulus over MPP/LDPE. Percent elongation of DNA/LDPE was higher than that of MPP/LDPE in the range of 5% – 30%. At the highest loading level, DNA/LDPE showed a mild decrease relative to MPP/LDPE (**Figure 7B**). Loading levels of intumescent flame retardants typically range between 15-25% in many commercial polymer applications, and DNA/LDPE has shown promising mechanical performance in this range due to improved dispersion into the LDPE substrate. Flammability testing was conducted according to UL-94 horizontal burn protocol. DNA/LDPE demonstrated a markedly reduced burn distance over MPP/LDPE at loading levels above 5% (**Figure 8A**). DNA did not appear to have an effect on flame spread rate (FSR), likely owing to the relatively short burn distances over which FSR was measured (**Figure 8B**).

Figure 7. Mechanical performance of compounded materials. **(A)** Tensile modulus and **(B)** percent elongation of DNA/LDPE and MPP/LDPE.

Figure 8. FR performance of compounded materials. (A) Percent burn distance and (B) flame spread rate of DNA/LDPE and MPP/LDPE based on UL-94 HB protocol. Char residue from DNA/LDPE (C) and MPP/LDPE (D) before and after the UL-94 horizontal burn test.

The greatly reduced burn distances observed in DNA/LDPE compounds are likely attributed to the early onset of DNA decomposition as seen via TGA (**Figure 5A**). This improved FR performance may also be a result of the deoxyribose units in DNA acting as a carbon source, facilitating stronger char formation over the native MPP formulation. The char residue observed for DNA/LDPE had a solid plastic consistency that formed rapidly and retained these properties following extinguishment (**Figure 8C**). The char

residues for MPP/LDPE possessed a more typical dry, brittle “carbon char” character (**Figure 8D**). FT-IR analysis showed strong absorption peaks at ~ 2900 , 1460 , and 717 cm^{-1} , in the DNA/LDPE char that are not observed in neat DNA (**Figure 9A,B**). These peaks correspond to the presence of C–H groups retained from the LDPE substrate. Conversely, MPP/LDPE char spectra did not appear to retain any original peaks from either MPP or LDPE (**Figure 9C,D**). The greatly reduced burn distance and increased substrate decomposition observed in DNA/LDPE samples suggest that DNA may be acting more rapidly than MPP in forming a char residue following ignition.

Figure 9. FT-IR characterization of LDPE, DNA (**A,B**), MPP (**C,D**) and corresponding char residues of compounded materials. Black: LDPE, red: DNA or MPP, green: compounded material (40%), blue: char of compounded material.

It is clear that DNA imparts strong FR properties to LDPE. Based on initial DSC studies, it is also evident that as a result of processing with LDPE at 145 °C, DNA undergoes certain changes that involve, at the minimum, denaturation of its two strands. However, it is likely that DNA also undergoes other biochemical changes, as is evidenced by the drastic color change from white DNA powder to brown DNA/LDPE blended material following compounding (**Figure 8C**). In order to determine if any of these changes may have an effect on FR properties, as well as to further understand the thermal degradation mechanism of DNA, thorough biochemical characterization of both neat and heat-treated DNA was performed (**Figure 10**). The heat-treatment of DNA involved heating neat DNA in the solid state to 145 °C, in order to replicate the temperature at which it was processed with LDPE. Size determination using agarose gel electrophoresis showed a distinct band corresponding to an average size of native DNA of less than 100 base pairs (bp). This is in good agreement with the expected average size of DNA starting material, reported to be an average of 50 bp (**Figure 10A**). On the other hand, heat-treated DNA could not be visualized on the gel, likely as a result of denaturation and chemical decomposition of its two strands, preventing the intercalation of ethidium bromide necessary for visualization. Additional characterization using size exclusion chromatography (SEC) revealed a major peak around an elution volume of 10 mL, at the void volume of the column, corresponding to intact, double-stranded DNA (**Figure 10B**). In the case of heat-treated DNA, this major peak is shifted toward 15 mL, with the reduction in size corresponding to single-stranded DNA as a result of denaturation. Furthermore, several lower-molecular weight peaks can be observed to

appear in the SEC tracing of heat-treated DNA, most notably at 22, 27, 44, and 60 mL likely corresponding to individual free nucleotides and single-stranded DNA oligomers. These findings confirm that at 145 °C, while DNA does not undergo any thermal weight loss, it does undergo complete denaturation, and partial fragmentation.[36]

Figure 10. Biochemical characterization of native and heat-treated DNA. **(A)** Agarose gel, Lane 1: DNA Ladder, Lane 2: native DNA, Lane 3: heat-treated DNA; **(B)** FPLC chromatogram, absorbance at 280 nm. **(C)** Scheme of the thermal oxidation of guanine to 8-oxo-guanine; **(D)** Mass spectra; **(E)** UV-vis spectra; **(F)** FT-IR spectra; **(G)** DSC thermograms.

Additional characterization was also performed in order to determine if any major chemical changes are occurring as a result of heat treatment. Previous reports indicate that nucleotide oxidation processes can occur at relatively low temperatures, such as the oxidation of guanine to 8-oxo-guanine (**Figure 10C**), a common biological indicator for oxidative damage.[36–38] Although many similar oxidation reactions are possible, the oxidation of guanine to 8-oxo-guanine is relatively well characterized, and can be used as a marker for similar oxidation events that may be occurring within the same temperature range. Analysis via mass spectroscopy (MS) of neat DNA revealed a distinct peak at a mass of 334.62 Da, corresponding to guanosine monophosphate (GMP). In the case of heat-treated DNA, this peak was significantly less abundant, and instead a new peak at a mass of 347.70 Da was seen, corresponding to 8-oxo-guanosine monophosphate (8-oxo-GMP) (**Figure 10D**). Additionally, UV studies of both native and heat-treated DNA revealed that while the general shape of the peak centered at 260 nm remained similar, a slight redshift and peak broadening suggest that oxidation processes are occurring, as previously reported (**Figure 10E**).[39] In addition, analysis via FT-IR spectroscopy showed peak broadening at both 1178 and 922 cm^{-1} , also indicative of oxidation (**Figure 10F**). Finally, since DNA undergoes oxidation in this

temperature range, DNA was analyzed via DSC under both air and nitrogen to determine if oxygen composition has an effect its endothermic/exothermic behavior. However, no difference was seen between the DSC traces (**Figure 10G**). As a result of these studies, it is concluded that DNA undergoes denaturation, partial fragmentation, and oxidation processes as a result of heating to 145 °C. Although these processes significantly modify the structure of DNA, they are less likely to reduce the composition of phosphate and amine groups necessary to impart FR properties to a polymer substrate.

4. Conclusion

This study has established DNA as a potent flame retardant additive for applications in melt-compounded low-density polyethylene (LDPE). When compared to melamine polyphosphate (MPP), one of the industry standard intumescent FR additives for plastics, DNA showed significantly improved processing compatibility with the LDPE matrix, drastically reduced burn distances in the horizontal configuration, early-onset solid char formation, and enhanced tensile properties at higher loading levels. Biochemical characterization of DNA in the solid state revealed that when heat-treated to its processing temperature of 145 °C, DNA undergoes denaturation, fragmentation, and oxidation, yet these processes do not appear to have an effect on its flame retardant properties. Since DNA has recently been shown as an effective FR coating and additive for textile and specialty polymer applications, this study serves to further understand the processing compatibility of DNA, its thermal degradation mechanism,

and expands the field of DNA-based FR applications into broad-use commodity polymers.

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Compatibility

Torque Reduction

Burn Distance Reduction

Char Formation