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Thermoplastic elastomers containing antimicrobial and antiviral additives for mobility applications

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ABSTRACT

The transmission of the SARS-CoV-2 coronavirus has been shown through droplets generated by infected people when coughing, sneezing, or talking in close contact. These droplets either reach the next person directly or land on nearby surfaces. The objective of this study is to develop a novel, durable, and effective disinfecting antimicrobial (antiviral, antibacterial, and antifungal) styrene-ethylene/butylene-styrene (SEBS) based thermoplastic elastomers (TPE). TPE incorporated with six different formulations was investigated for mechanical and antiviral performance. The formulations consist of a combination of zinc pyrithione (ZnPT), sodium pentaborate pentahydrate (NaB), disodium octaborate tetrahydrate (DOT), and chlorhexidine (CHX). ZnPT and DOT incorporated TPE showed a reduction of microbes such as bacteria by up to 99.99%, deactivated Adenovirus, Poliovirus, Norovirus, and reduced a strain of the coronavirus family by 99.95% in 60 min on TPE samples. Control samples had higher tensile strengths among all formulations and tensile strength decreased by around 14%, 21% and 27% for ZnPT and DOT combinations compared to control samples. The elongation at break decreased by around 7%, 9% and 12% with ZnPT and DOT combinations, where it reached minimum values of 720%, 702% and 684%, respectively. The 100% Modulus and 300% Modulus slightly increased with ZnPT and NaB combination (reaching values from 1.6 to 1.9 MPa and 2.6–2.9 MPa respectively) in comparison with control samples. The MFI also decreased with antimicrobial and antiviral additives (decreasing values from 64.8 to 43.3 g/10 min). ZnPT and NaB combination showed the lowest MFI (43.3 g/10 min) and reduced the MFI of control sample by around 33%. TPE samples containing ZnPT and DOT combination showed biocidal activity against the microorganisms tested and can be used to develop antimicrobial products for multiple touchpoints within a vehicle and micro-mobility.

1. Introduction

Despite the efforts to reduce the spread of Covid-19, the number of cases are still raging worldwide, and new virus strains are reported globally [1]. The pandemic has changed our lives in significant ways and will continue to impact daily life activities. One example is how the public perceives the risk associated with using shared mobility or public transportation. In 2020, several surveys were conducted to understand a change in consumer behaviors and interests during the Covid-19 pandemic [2,3]. These studies focused on automotive features that can potentially prevent the spread of infectious pathogens. It was highlighted that more than half of the survey participants were concerned about infecting themselves while participating in ridesharing. As public transportation and ridesharing were perceived as the most likely place to get infected, antimicrobial vehicle interior surfaces were perceived as one of the ways to increase consumer confidence in the shared mobility.

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, yeast, algae or viruses [4]. This term encompasses the terms antiviral, antibacterial and antifungal substances, which refer directly to inactivate or kill viruses, bacteria and...
fungi, respectively. Several attempts have been made to identify the transmission pathways of infectious microbes in public transport and vehicle interiors [5–9]. Stephenson et al. demonstrated which frequently touched surfaces in a vehicle have a higher number of contaminations with Staphylococcus aureus. S. aureus was tested due to its prevalence in public domain and ability to cause a variety of infectious diseases. Staphylococcus epidermidis, S. aureus, and Staphylococcus warneri were shown to be the most prevalent microbes tested making up 43, 31, and 13% of the staphylococcal isolates [5]. Different interior locations were swabbed and analyzed for bacterial colonization. Steering wheel, center console, door handle, windows switches, and gear shifters were found to be the most highly colonized locations that correspond well with the areas that are frequently touched by the occupants. Teresa et al. assessed bacterial contamination on frequently touched surfaces in public buses [9]. They further studied the transmission of the pathogens from surfaces to the hands of passengers indicating potential routes for the spread of the infection.

Although the CDC’s latest guideline states that the risk of getting infected with SARS-CoV-2 through touching contaminated surfaces is generally low [10], earlier studies have shown that the virus can survive for up to 3 days on plastic surfaces [11,12]. Scientists warn that the transmission rates are much higher indoors and depend on the number of infected individuals present in a closed environment [13–15]. Because a car environment has an inherent risk of being in a confined space, there is a huge drive to develop antimicrobial protection in automobiles. It is an urgent priority to prepare antimicrobial materials for commonly touched areas, so that they may provide superior protection against diverse types of viruses and bacteria.

There are several types of antimicrobial materials that are proven to be effective in preventing the spread of infectious microorganisms [16, 17]. Metal nanoparticles such as silver, copper, and gold have historically been widely known for antimicrobial properties. Besides the metal nanoparticles, zinc pyrithione (ZnPT) and boron compounds are well-known biocide agents for polymers. Pitt et al. evaluated the antimicrobial potential of styrene-ethylene/butylene-styrene (SEBS) based thermoplastic elastomers (TPE) incorporated with ZnPT and silver nanoparticles [18]. Samples prepared with zinc pyrithione eliminated 99.9% of the Escherichia coli and 99.7% of the S. aureus population and presented an inhibition zone in the bactericidal assay. Iyigundoglu et al. studied that the modification of cotton fabrics with 3% sodium pentaborate pentahydrate (NaB), disodium octaborate tetrahydrate (DOT) and chlorhexidine (CHX) in inhibiting the growth of microbial strains. This study describes: (a) the mechanical and physical characteristics of antimicrobial agents-incorporated TPE compounds, (b) the bactericidal performance of incorporated TPE compounds against Gram-positive (S. aureus, Methicillin Resistant S. aureus (MRSA)) and Gram-negative bacteria (E. coli), (c) the fungicidal performance of incorporated TPE compounds against the yeast Candida albicans and filamentous fungi Aspergillus niger, (d) the virucidal performance of incorporated TPE compounds against DNA (Adenovirus) and RNA viruses (Bovine coronavirus, Poliovirus, Norovirus). The leading antimicrobial-treated TPE polymer will be further implemented into handlebar grips for micro mobility and inserts in console bin and floor mats in a vehicle. Previously reported antimicrobial materials have focused on antibacterial and antifungal capabilities; however, there has been much less focus on antiviral surfaces. Here we aim to evaluate the antiviral effectiveness of the antimicrobial-treated TPE polymers as well as mechanical, physical properties, odor, and long-term durability (heat aging) which is essential requirements for micro-mobility and transportation industry. This article evaluates the antimicrobial potential of TPE compounds incorporated with ZnPT, sodium pentaborate pentahydrate (NaB), disodium octaborate tetrahydrate (DOT) and chlorhexidine (CHX) in inhibiting the growth of microbial strains. This study describes: (a) the mechanical and physical characteristics of antimicrobial agents-incorporated TPE compounds, (b) the bactericidal performance of incorporated TPE compounds against Gram-positive (S. aureus, Methicillin Resistant S. aureus (MRSA)) and Gram-negative bacteria (E. coli), (c) the fungicidal performance of incorporated TPE compounds against the yeast Candida albicans and filamentous fungi Aspergillus niger, (d) the virucidal performance of incorporated TPE compounds against DNA (Adenovirus) and RNA viruses (Bovine coronavirus, Poliovirus, Norovirus). The leading antimicrobial-treated TPE polymer will be further implemented into handlebar grips for micro mobility safety. Details of the performance evaluation will be reported in a subsequent publication.

2. Experimental

2.1. Microorganism, viruses, and cell lines

In this study, six different antimicrobial combinations are blended with SEBS-based TPE to create six potential antimicrobial substances. The lists of microbial and viral species are given in Tables 1 and 2. Each sample was tested against various bacteria, yeast, fungi, and viruses, to determine which has the superior antimicrobial properties. The bacteria, yeast and fungi species were obtained from the American Type Culture Collection (ATCC). Viruses and their cell lines obtained from ATCC and FLI The Riems Virus Bank (RVB).
Table 1
The list of microbial species tested in antimicrobial studies.

| Bacteria                     | Yeast                       | Fungi                      |
|------------------------------|-----------------------------|----------------------------|
| Escherichia coli (ATCC 8739) | Candida albicans            | Aspergillus niger          |
| Staphylococcus aureus (ATCC 6538) | (ATCC 10231)               |                            |
| Methicillin-resistant Staphylococcus aureus (MRSA) (ATCC 33529) |                             |                            |

Table 2
The list of viral species and cell lines used in antiviral studies.

| Virus                          | Cell Line                      |
|--------------------------------|--------------------------------|
| Human adenovirus type 5 (ATCC VR-5) | HEP-2 (ATCC CCL-23)            |
| Bovine Coronavirus (ATCC VR-874) | MDRK (ATCC CCL-22)             |
| Human poliovirus type 1 (RVB-1260) | HeLa (ATCC CCL-2)              |
| Murine norovirus (RVB-0651)    | RAW (ATCC TIB-71)              |

2.2. Chemicals

Disodium octoborate tetrahydrate (Na₂B₉O₁₃·4H₂O) (DOT) was obtained from Eti Maden Operations (Ankara, Turkey). Zinc pyrithione (C₈H₆N₂O₅·Zn) (ZnPT) and Sodium pentoborate pentahydrate (NaB₉O₆·5H₂O) (NaB₉O₆·5H₂O) (NaB₉O₆·5H₂O) (NaB₉O₆·5H₂O) were purchased from local suppliers. Chlorhexidine (C₁₂H₁₆Cl₅N₈O₂) (CHX) was purchased from Sigma-Aldrich. TPE component, polypropylene (PP) homopolymer named HOPELEN Y-130, having 4 g/10min. MFI, 35 MPa tensile strength and 65 Shore D hardness values, was supplied from Lotte Chemical Company. SEBS having solution polymerization, SEBS block copolymer, was supplied from TSRC Corporation, paraffinic oil with the viscosity of 100 cSt at 40 °C. The additives (coloring agent and antioxidant) were included at a proportion of 3% by weight (as recommended by the supplier) in a TPE formulation compounded by SEBS, PP, white mineral oil, at the ratio of 30/20/50, respectively. Nutrient agar (NA), potato dextrose agar (PDA), phosphate buffered saline (PBS) were purchased from Sigma-Aldrich. All chemicals which were used in this study were of technical grade and high purity level.

2.3. Antimicrobial polymer production through melt compounding

The mixture of evenly proportioned antimicrobial additives and TPE was extruded using a Twin-Screw Extruder, Coperion ZSK 32 that contains 14-barrel sections at a screw speed of 550 RPM. Screw diameter of the extruder, L/D ratio and throughput was 32 mm, 44 and 10–200 kg/h, respectively. All main ingredients were fed from barrel 1 shown in Fig. 1, that is the main feeding zone. The barrel temperature was set to 50 °C in order to avoid agglomeration and premature melting of materials. Residence time was also measured as 150 s. While atmospheric vent was located at barrel 6, vacuum was linked at barrel 13. Two separate identical kneading block clusters were located at the length corresponding to barrel 3,4,5 and barrel 7,8,9. Each kneading block clusters consisted of 9 kneading blocks having 5 bi-lobal kneading elements with 45°. The total length of each kneading block was 42 mm. The rest of the screw elements were conveying elements. Melt temperature is a critical property in controlling the extrusion process and optimizing the throughput while minimizing polymer, filler, and additives degradation. At the hot extrusion temperatures of 160–190 °C, the samples extruded without any defects such as degradation, tearing, and blister. TPE formulation was fed into a hopper along with antimicrobial and antiviral additives that feeds down into the extruder through main feeding. The mixture pass through the barrel on the screw while being heated to the melting temperature of 160–190 °C. At the end of the screw, the antimicrobial compound flows across a screen and a breaker plate which removes any contaminants or inconsistencies in the plastic and changes the motion of the plastic from rotational to longitudinal [25]. After the passing this stage, the compound is ready for the die which is designed so that the plastic flows smoothly and evenly from the cylindrical profile of the extruder into the final profile shape [25]. There is also lack of information available on the effect of processing temperature on the mechanical properties of antimicrobial and antiviral-containing compounds. This fact inspired us to study the effect of processing temperature on mechanical and thermal properties of antimicrobial and antiviral-containing compounds in our next publication. Details of thermal stability and degradation of neat polymer and antimicrobial and antiviral additives and compounds will be investigated with thermogravimetric analysis (TGA). The extruded material was collected and allowed to cool for 5 min. To convert the melt form to pellet, underwater pelletizing system were used.

After each sample had been extruded. The samples were then injection molded using a ENGEL Victory 90 injection molding machine. The parameters of injection molding are represented in Table 3. The thin square plaques have a dimension of 130 × 130 × 2 mm. The dog bone specimens were prepared according to ISO 37 with Type I. The formulation that was designed to investigate the effect of antimicrobial additives on TPE is represented in Table 4.

2.4. Characterization of the antimicrobial compounds

Areal density of the specimens was measured in accordance with the ISO 1183 1-A by immersing in water at 23 °C. A metal sinker was also used since the materials had density lower than water. At least 5 readings were taken for each material ensuring a coefficient of variation of less than 0.1%. MFI was measured by flowing out the polymer melt from

Table 3
Injection molding parameters.

| Injection Molding Parameters           | Square Plaque | Thick Plaque |
|----------------------------------------|---------------|--------------|
| Barrel Temperature profile (°C)        | 180–185-185   | 180–185-185-190 |
| Mold Temperature (°C)                  | 25            | 25           |
| Injection Speed (mm/s)                 | Stage 1: 25 (until 20% of shot volume) | Stage 2: 25 (until 40% of shot volume) |
|                                        | Stage 3: 25   | Stage 4:      |
|                                        | (until 60% of shot volume) |Stage 5: 45 (until 80% of shot volume) |
|                                        | 55 (until 100% of shot volume) | 55           |
| Injection Pressure (bar)               | 60            | 56           |
| Pack-hold Pressure (bar)               | 30            | 55           |
| Pack-hold Time (s)                     | 20            | 20           |
| In-mold Cooling Time (s)               | 30            | 25           |
| Back Pressure (bar)                    | 5             | 5            |
| Screw Rotation Speed (mm/s)            | 40            | 40           |

Fig. 1. Schematic representation of extruder design temperatures of corresponding extruder zones.
a standard die (2.095 × 8 mm) at a 190 °C and with a standard weight of 5 kg applied to the piston. At least five specimens were tested for each formulation. Zwick Roell Z010 machine was used for the characterization of tensile properties of the TPE based specimens. Tests were conducted at a speed of 500 mm/min in accordance to ISO 37. Tensile strength at break, elongation at break, 100% modulus and 300% modulus were calculated. Compression set test aims to determine the plastic and elastic deformation behavior. This test was performed according to ISO 815, Method B with 25% comparison. The test samples were cut with the thickness and diameter were 6 ± 0.2 mm and 13 ± 0.2 mm, respectively. Prepared samples were compressed and aged at three different conditions (72 h/23 °C, 22 h/70 °C and 22 h/100 °C) and measured before and after the aged conditions. Hardness of the specimen was measured using a Gobritore Shore A Hardness Tester on 6 mm thick plaques as per ISO 7619-1. During the test, a 5 kg of force was applied for 3 and 15 s with the needle at the tip of the instrument. Hardness was measured from three different locations of the plaque. For heat aging measurements, test samples cut according to ISO 37 standard were aged for 7 days and 150 h in the oven. Tensile tests were conducted before and after aging via Zwick Roell Z010. At least six tensile, compression and hardness specimens were tested for each formulation.

2.5.1. Preparation of microbial inoculums

Thermal properties of the material were investigated using differential scanning calorimetry (Mettler-Toledo DSC 3) in accordance to ISO 11357–3. Specimens with a mass in the range of 8–12 mg were placed in aluminum pans and sealed. The scanning protocol for all the specimens were as follows: heat from 25 °C to 200 °C at 40 °C/min, hold at 200 °C for 5 min, cool from 200 °C to −80 °C at 40 °C/min, hold at −80 °C for 5 min then heat from −80 °C to 200 °C at 40 °C/min. Samples were held at −80 °C to stabilize the temperature and clearly find the Tg of the TPE and compounds which were around −55 °C. The first cycle of heating is performed to erase all thermal history and residual stresses induced during injection molding. Tests were carried out under Nitrogen environment at a gas flow rate of 50 mL/min. Three samples, randomly selected for each formulation, were used for the DSC measurement.

Odor evaluation was completed using samples cut into 5.08 cm by 2.00 mm by 12.7 cm and conditioned in a sealed glass jar for 24 h at 40 °C. Five panelists evaluated and ranked the perceived odor of the samples on a scale from 1 to 6, according to Ford’s internal performance specification. Rating of 1 is a “non-perceptible” smell and goes to 6 which is “extremely disturbing.” [26] To be accepted for automotive application, a component must be rated less than 3.5 correlating to a smell that is “intense enough to be slightly disturbing.”

2.5. Antimicrobial studies

2.5.1. Preparation of microbial inoculums

Microorganisms specified in Table 1 were transferred from stock culture to slant culture medium (NA, and PDA) and incubated at 24 h for bacteria at (35 ± 1) °C, 48 h for yeast at (35 ± 1) °C and 72 h for fungi at (30 ± 1) °C. Approximately 10^6 CFU/ml and 10^8 spores/mL microbial suspensions were prepared and directly used for the agar diffusion method. For the ISO 22196 method, microbial suspensions were diluted to approximately 10^8 CFU/mL in NA, and PDA.

2.5.2. Agar diffusion method

In order to reduce the test numbers, the antimicrobial activity of all formulations was investigated with the agar diffusion method. The antimicrobial activity of surfaces was investigated against microorganisms given at Table 1. 100 μL of fresh suspensions prepared as stated in part 2.5.1 were spread on NA for bacteria, PDA for yeast and fungi species. Polymer samples with active agents and control groups were cut into the approximate size of (20 mm × 10 mm) and placed onto inoculated mediums. The inoculated plates were incubated 24 h for bacterial strains, 48 h for yeast and 72 h for fungi species. The antimicrobial activity of agar diffusion assay was evaluated by observing the presence of clear zones around test specimens. Antimicrobial activity tests were performed in duplicate.

2.5.3. Measurement of antimicrobial activity on TPE

Antimicrobial activities on TPE were evaluated based on ISO 22196 standard method. The bacterial, yeast and fungal species used for antimicrobial activity tests are given in Table 1. Flat control and antimicrobial TPE surfaces were cut into equal sizes of (50 ± 2) mm × (50 ± 2) mm and sterilized by wiping with 70% (v/v) ethanol then placed into sterile Petri dishes. 400 μl of test inoculums prepared in section 2.5.1 were pipetted onto TPE test samples. Test inoculums were covered with 40 mm × 40 mm sterilized polypropylene (PP) films to spread the inoculum to the edges, and lids of Petri dishes were replaced. Antimicrobial activities of TPE samples were determined by measuring the survival of microorganisms held in contact for 24 h bacteria at (35 ± 1) °C and yeast and fungus for 48 h at (30 ± 1) °C under a relative humidity of above 90%. Untreated TPE surfaces were used as control groups for each microorganism. Microorganisms were recovered from polymer samples by adding 10 mL soybean casein digest broth with lecithin and polyoxyethylene sorbitan monooleate (SCDLP broth) prepared as described in the standard. SCDLP broth containing inoculum was diluted by 10-fold serial dilutions in PBS and inoculated onto plate count agar. After incubation at (35 ± 1) °C for 48 h, colonies were counted, and microorganism counts were calculated as log CFU/sample [27].

2.5.4. Antiviral activity

Adenovirus (AdV), Bovine Coronavirus (BCoV), Poliovirus (PoV), and Murine norovirus (MNV) species specified at Table 2 were used for antiviral studies. Infected cell cultures were prepared as described previously [28]. The antiviral properties of the TPE samples were investigated according to the ISO 21702:2019 standard. Flat TPE samples were cut into equal sizes of (50 ± 2) mm × (50 ± 2) were cleaned by wiping with 70% (v/v) ethanol. Virus concentration was adjusted to approximately 10^3 plaque-forming units (PFU) per mL and 400 μl virus suspension pipetted onto the sterilized surfaces and test inoculums were covered with PP films and placed in Petri dishes. Samples were kept at incubation at 25 °C under a relative humidity of above 90% for 1 min, 5 min, 10 min, 30 min, 60 min, and 120 min. Inoculated surfaces were then washed with 10 mL SCDLP to recover the viruses.

Next, 225 μl of maintenance medium described in ISO 21702 standard was added to the 96 well plate. Serial 10-fold dilutions of the recovered virus were prepared in the first eight well. The cells given in Table 2 were growth as monolayer in another 96 well plate and used for the plaque-forming units (PFU) per mL and 400 μl virus suspension pipetted onto the sterilized surfaces and test inoculums were covered with PP films and placed in Petri dishes. Samples were kept at incubation at 25 °C under a relative humidity of above 90% for 1 min, 5 min, 10 min, 30 min, 60 min, and 120 min. Inoculated surfaces were then washed with 10 mL SCDLP to recover the viruses.

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the viruses can be absorbed by the cells. The plates were moved back and forth for every 15 min to have a homogeneous viral distribution in the cells. Next, the cells were washed with maintenance medium and 100 μl maintenance medium containing 92% (v/v) PBS were placed on each well. Infected cells were incubated for 4–6 days at 35 °C for BCoV and 2–4 days in at 37 °C for AdV, PoV, and MNV in 5% CO2 atmosphere. At the end of the incubation, the cytopathic effect (CPE) due to the viral infection is evaluated under an inverted microscope. Viral titers in the cell culture were calculated by Spearman–Karber method [29].

The antiviral activity was calculated using the formula

\[ R = \frac{(U_t - U_0) - (A_t - A_0)}{U_t - A_t} \]

Where the antiviral activity is represented by \( R \), the average of common cells. Next, the cells were washed with maintenance medium and 100 μl PBS were placed back on each well. Infected cells were incubated for 4 min, 5 min, 10 min, 30 min, and 60 min, respectively. From these untreated test specimens after 1 min, 5 min, 10 min, 30 min, and 60 min is represented by \( U_t \), from three untreated test specimens after 1 min, 5 min, 10 min, 30 min, and 60 min is represented by \( A_t \).

3. Results and discussions

3.1. Mechanical and physical properties

The results of the mechanical and physical property testing are shown in Table 5. As expected, maximum increase of around 10% was found for Sample 6 with the highest amount of additive, and the least increase in density of around 3% was reported for Sample 1 with the lowest amount of additive. Similarly, hardness also increased after the addition of antiviral additives. Hardness increased by around 10% for Sample 6 and the least affected was Sample 1 with an increase of around 3%. Increase in hardness might negatively impact customer experience if these materials are used for applications where the user is actively in contact with this material throughout the period of operation.

Inclusion of antiviral additives resulted in brittle specimens with slightly higher tensile modulus and lower tensile strength and lower elongation at break. The elongation at break decreased by around 7%, 9% and 12% with ZnPT and DOT combinations, where it reached minimum values of 720%, 702% and 684%, respectively. The 100% Modulus and 300% Modulus slightly increased with ZnPT and NaB combination (reaching values from 1.6 to 1.9 MPa and 2.6–2.9 MPa respectively) in comparison with control samples. The tear strength perpendicular to flow was not affected for most of the specimens. Interestingly, the specimen whose tear strength was reduced significantly had one of the least amount of additive (Sample 1). Control samples had higher tensile strengths among all formulations and tensile strength decreased by around 14% and 27% for sample 1 and sample 3 compared to control samples, respectively. Similar results also reported by Veranitsagul et al. and Silva et al. in terms of tensile strength of nanosilver-coated carbon black samples with PBS compounds and calcium oxide (CaO) nanoparticles samples with LDPE [23,24]. The addition of high amount of NaB (up to 15% by wt.) in sample 6 resulted in marginal effect in tensile strength and elongation at break suggesting NaB most likely acts as a non-reinforcing filler in the composite [28]. The surface modification of the antimicrobial and antiviral additives or using special coupling agent may improve the interaction with the polymer matrix and the dispersion and mechanical properties. Antimicrobial properties and processing conditions are the critical parameters for the targeted applications. This study showed the antimicrobial activity of ZnPT-DOT incorporated TPE materials against all the fungal, yeast, bacterial and viral species tested. No change or modification on tool and processing parameters for the Sample 1. The difference between antiviral TPE and control sample was marginal in terms of processing. Therefore, Sample 1 was selected for the long-term durability testing and molding trials which will be discussed in the forthcoming publication.

The MFI also decreased with antimicrobial and antiviral additives (decreasing values from 64.8 to 43.3 g/10 min). MFI of sample 1 (63.5 g/10 min) and control sample (64.8 g/10 min) were similar to each other. Sample 6 showed the lowest MFI (43.3 g/10 min) and reduced the MFI of control sample by around 33% suggesting that the addition of additives significantly increased the viscosity of the polymer melt. Maerker and Sinton demonstrated an increase in viscosity with the addition of sodium borate in poly vinyl alcohol (PVA) solution over a wide range of shear rates [31]. In another study, Inoue and Osaki observed a shear thickening behavior when sodium borate was added to polymers like PVA [32]. Higher viscosity is associated with higher torque and consequently more energy is needed for processing the polymer melt.

The melting point was taken as the main peak of the endothermic curve. The melting point of neat TPE was 155 °C. The effect of antimicrobial and antiviral additives on Tm of the compounds is presented in Table 5. It can be seen from Table 5 that the melting points of compounds were between 154 °C and 155 °C. The addition of the antimicrobial and antiviral additives to compounds did not have significant influence on the Tm of the specimens. This study illustrates that adding antimicrobial and antiviral materials does not change the Tm of the

### Table 5

| Characterization of TPE-based antimicrobial compounds. | Standard | Control | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
|-------------------------------------------------------|----------|---------|----------|----------|----------|----------|----------|----------|
| Densiy (g/cm3)                                        | ISO 1183 1-A | 0.89 (0.001) | 0.92 (0.002) | 0.94 (0.001) | 0.95 (0.001) | 0.96 (0.001) | 0.95 (0.002) | 0.98 (0.001) |
| Melt Flow Index (g/10min) (190°C, 5 kg)               | ISO 1133 | 64.8 (2.1) | 63.5 (1.9) | 58 (2.3) | 59.2 (2.0) | 55.6 (2.5) | 52.2 (2.8) | 43.3 (2.1) |
| Hardness - 6 mm plaka (SHORE A)                       | ISO 7619-1 (15 s) | 59 (0.4) | 61 (0.3) | 62 (0.4) | 64 (0.5) | 64 (0.3) | 62 (0.3) | 65 (0.3) |
| 100% Modulus (MPa)                                    | ISO 37, DIN | 1.6 (0.01) | 1.7 (0.03) | 1.7 (0.01) | 1.8 (0.04) | 1.7 (0.07) | 1.8 (0.05) | 1.9 (0.01) |
| 300% Modulus (MPa)                                    | ISO 37, DIN | 2.6 (0.01) | 2.8 (0.05) | 2.7 (0.08) | 2.8 (0.02) | 2.7 (0.05) | 2.8 (0.06) | 2.9 (0.02) |
| Tensile Strength at Break (MPa)                       | ISO 37 | 9.6 (0.013) | 8.3 (0.014) | 7.6 (0.011) | 7.0 (0.017) | 7.5 (0.015) | 8.1 (0.019) | 7.4 (0.011) |
| Elongation at Break (%)                               | ISO 37, DIN | 775 (10.3) | 720 (10.2) | 702 (8.2) | 684 (10.2) | 724 (9.3) | 731 (10.2) | 691 (10.3) |
| Tear Strength (Perpendicular to flow) (N/mm)          | ISO 341 | 34.8 (0.01) | 30.8 (0.02) | 33.7 (0.01) | 33.7 (0.02) | 32 (0.03) | 33.7 (0.01) | 33.1 (0.02) |
| DSC - Tg (°C)                                         | ISO 11357-2 | –56.07 (0.2) | –55.9 (0.1) | –54.91 (0.2) | –57.62 (0.1) | –55.09 (0.3) | –60.06 (0.2) | –55.93 (0.2) |
| DSC - Ta (°C)                                         | ISO 11357-3 | 155.24 (0.3) | 154.72 (0.2) | 154.92 (0.2) | 154.07 (0.1) | 153.94 (0.2) | 154.55 (0.3) | 154.23 (0.2) |
| DSC - Td (°C)                                         | ISO 11357-3 | 103.5 (0.1) | 107.91 (0.2) | 107.16 (0.1) | 107.67 (0.1) | 106.64 (0.2) | 106.53 (0.2) | 108.17 (0.2) |

Parentheses indicate standard deviation.
compounds or it has a marginal effect at most. From Table 5, it can be concluded that the $T_g$ and $T_m$ values of the compounds were strongly influenced by the matrix polymer. The relative shift of $T_g$ was quite evident for all the samples. The higher $T_g$ of the TPE compounds compared to the unfilled TPE means that upon cooling, crystallization of TPE begins earlier in the compounds than in the unfilled TPE as antimicrobial and antiviral additives act as nucleating agents to induce crystallization of the polymer melt in the cooling process [33].

3.2. Agar diffusion test

The six formulations underwent an agar diffusion test against Gram-positive (S. aureus, Methicillin Resistant S. aureus (MRSA)), Gram-negative bacteria (E. coli), yeast (C. albicans), and fungus (A. niger). Agar diffusion test results are given in Table 6. Due to the differences in polymer sizes, inhibition zones were not measured quantitatively. Clear zones around the polymers were specified as positive, if there is no clear zone antimicrobial activity evaluated as negative. All samples except Sample 4 when tested against S. aureus, showed antibacterial activity. Sample 4 was the only composition made of CHX.

3.3. Mechanical properties after heat aging testing

A baseline of performance characteristics needs to be maintained when developing novel materials to ensure quality. With the addition of antimicrobial fillers, the control samples were used as that benchmark to ensure the filler’s addition either maintained or improved the TPE’s performance. As such, formulations were narrowed down throughout testing. Before progressing to the heat aging tests, the sample formulations were narrowed to Sample 1, 5, and 6. Out of these six formulations, Sample 2 and 3 were discarded based on cost and significant odor during the melt compounding process. Sample 4 was removed because of its ineffectiveness against S. aureus during the Agar diffusion testing. Samples 1, 5, and 6 underwent long-term tensile and compression testing. Sample 1 showed the highest change in tensile strength (15%), and the elongation at break (26%) after seven days at 150 °C in Table 7. The compression set at ambient (23 °C) and elevated temperature (70 °C) did not show any discrepancies among these three samples. Antimicrobial additives like ZnPT, which have been used in all formulations except Sample 4, are considered migratory in nature that can leach to the surface. More aggressive hygroscopic effect such as high temperature can accelerate the internal migration [34]. Higher release rate can result in higher efficacy for specimen that are subjected to ageing tests at elevated temperature. One of the inherent concerns of using these antimicrobial additives is their detectable odor during the process and use phase. The three samples underwent odor testing. The odor rating for Sample 1 is low compared to Sample 5 and 6. Since a rating of “3.5” or lower is considered acceptable for automotive interior applications, Sample 1 was used for further testing.

3.4. Antibacterial and antifungal properties

Samples 1, 5, and 6 were evaluated for antimicrobial activity following ISO 22196:2011 against bacteria, yeast and fungi. Table 8 summarizes the efficacy of antimicrobials in both Log reduction and percent reduction. All three samples performed at 99.9% reduction against the Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria even after undergoing typical melt compounding processing. Samples 1, 5, and 6 are all made up of ZnPT and either DOT or NaB. These results are consistent with the previously mentioned study by Pitta et al. that reported a 99.9% reduction of E. coli and 99.7% reduction against S. aureus on a TPE/ZnPT (1.5% by weight in a TPE formulation) compound [18]. ZnPT has also long been used in the cosmetic industry, working as an antimicrobial against gram-positive and gram-negative bacteria and fungus in commonly used products such as anti-dandruff shampoos [35,36]. It has also been used as an antifungal and antimicrobial agent in polyurethane foam, polyvinyl chloride (PVC), textiles, and paint [18]. ZnPT is understood to affect a cellular structure in several ways, including affecting the cells’ proton pumps which inhibit membrane transport, reducing intracellular ATP levels, and forming pseudoquaternary ammonium group [36]. The cell is then in a state of disorder, unable to properly export toxins or intake nutrients. Inside polymer matrices, organic additives such as ZnPT are known to migrate to the surface of the polymer, thus creating an inhibition zone of the surface of the product [18].

A study by Argin et al. on gelatin based packaging film reported that incorporation of both DOT and NaB showed an inhibitory effect on S. aureus, Pseudomonas aeruginosa, A. niger, and C. albicans (with higher additive concentrations) [37]. Results of antifungal activity is also presented in Table 8. A good inhibitory effect against the yeast C. albicans and filamentous fungi A. niger was obtained with the three combinations tested (ZnPT and either DOT or NaB). Two logarithmic reductions in the amount of cell growth were interpreted as the bactericidal or fungicidal effect of the TPE samples tested [38]. Following these results, the three

| Table 7 | Heat aging test results and odor characterization of TPE-based compounds. |
|---------|--------------------------------------------------------------------------|
|         | Standard | Control | Sample 1 | Sample 5 | Sample 6 | Sample 6 |
| Odor (Ford Test Method) | BO 131-03 | 2 | 2 | 4 | 4 |
| Change in Tensile strength (7 days/150 °C) (%) | ISO 188 | +12 | +15 | +2 (0.1) | +1 (0.1) |
| (2.0) |
| Elongation at break after ageing (7 days/150 °C) (%) | ISO 188 | +24 | +26 | +16 | +15 |
| (2.5) | (3.1) | (2.3) | (2.1) |
| Compression set (72 h/23 °C) (%) | ISO 815 | -21 | -20 | -19 | -19 |
| (2.5) | (2.2) | (2.3) |
| Compression set (22 h/70 °C) (%) | ISO 816 | -36.5 | -37.5 | -37.7 | -38.8 |
| (2.1) | (1.9) | (2.4) | (2.3) |

Parentheses indicate standard deviation.

| Table 8 | Antimicrobial activity results according to ISO 22196:2011 method. |
|---------|---------------------------------------------------------------------|
| Log reduction & | % reduction |
| Sample 1 | Sample 5 | Sample 6 | Sample 1 | Sample 5 | Sample 6 |
| E. coli | 3.69 | 3.54 | 3.68 | 99.98 | 99.97 | 99.98 |
| S. aureus | 3.84 | 3.30 | 3.74 | 99.99 | 99.95 | 99.98 |
| C. albicans | 3.60 | 3.65 | 3.47 | 99.97 | 99.98 | 99.97 |
| A. niger | 3.17 | 3.25 | 3.27 | 99.93 | 99.94 | 99.95 |

| Table 6 | Antimicrobial activity test results according to agar diffusion method. |
|---------|---------------------------------------------------------------------|
| Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Control |
| E. coli | + | + | + | + | + | – |
| S. aureus | + | + | + | + | + | – |
| MRSA | + | + | + | + | + | – |
| C. albicans | + | + | + | + | + | – |
| A. niger | + | + | + | + | + | – |
samples were tested for antiviral properties with a contact time of 60 min. Table 9 shows the Log and percent reduction of sample 1, 5, and 6 against BCoV, AdV, PoV, and MNV.

All samples reached a 99% reduction. Samples 1 and 5 obtained a 99.9% reduction against the BCoV. Sample 6 showed a 99.9% reduction against PoV. Iyigundogdu et, al. published findings on textiles with a NaB and triclosan solution, reduced the viral titers by 60% for both PoV and AdV [19]. Qui et, al. Reported that ZnPT inhibited Herpes Simplex Virus (HSV)-1 and –2 replication and viral gene expression [39]. At this point, the three Samples (1, 5, 6) were evaluated for physical and antiviral properties. While all three performed similarly in terms of antiviral efficacy, Sample 5 and 6 did not meet the odor requirements. Therefore, Sample 1 was selected as the leading formulation and progressed to more testing.

### 3.5. Antiviral properties after heat aging testing

In general, specific experimental conditions such as temperature and contact times are critical to defining the antiviral properties of polymers when comparing the virucidal activities of materials with and without viral loads for the intended applications [40].

Antiviral properties were reevaluated against BCoV, AdV, PoV, and MNV, after heat aging testing (7 days/150 °C) on Sample 1. Heat aging tests were done in accordance with procedures established by OEMs to represent long term durability. The short time and high temperature represent how the material would behave over a long period of time with extreme vehicle conditions. Table 10 shows the antiviral activity of Sample 1 after heat aging tests. The contact time was recorded at 5 and 60 min. Sample 1 showed a 99.97% reduction at 60 min against all four viruses. By increasing the temperature, the antiviral efficiency was raised as reported for zinc ions [41]. It is believed that the antimicrobial can migrate from the bulk to the surface of the plastic by heat aging. Once diffused to the surface, a virus might be precipitated, aggregated, or have its structure destroyed by zinc ions [42].

### 3.6. Antiviral properties at various contact times

In the mobility industry, an ideal antiviral material is one that reduces the viral load quickly, as there is potentially only a short window of time between different riders [43]. The antiviral activity of Sample 1 (ZnPT and DOT combination) was evaluated by comparing the survival of BCoV on antiviral TPE surfaces for several contact times (1, 5, 10, 30 and 60 min) at room temperature, reported in Table 11. At times 30,10, 5 and 1 min, the sample showed low (1.66, 1, 1 log10 and no) antiviral activity, respectively. At 60 min, there was a 99.95% reduction and high antiviral activity (3.33 log10). By increasing the contact time between the viruses and the sample, the antiviral efficiency was raised due to the combined effect of the antimicrobial materials chemistry (higher biocidal release from the sample surface affecting more of the viral viability) and dehydration of virus particles [44].

Based on these findings, Sample 1 (3.1% ZnPT and 2.88% DOT) was selected as the leading antimicrobial coating and progressed to molding trials and then application-use-tests such weathering, sweat exposure, and abrasion. These findings will be further evaluated in a future publication.

### 4. Conclusion

This study showed the antimicrobial activity of ZnPT-DOT incorporated TPE materials against all the fungus, yeast, bacterial and viral species tested. Inclusion of antiviral additives resulted in brittle specimens with slightly higher tensile modulus and lower tensile strength and lower elongation at break. To the best of the authors’ knowledge, there are currently no automotive standards for antimicrobial performance. Many material suppliers are working on developing antimicrobial products but do not always specify test methods and conditions. Because many factors can affect the efficacy of antimicrobial-treated materials (e.g., test species, inoculum concentration, exposure time), it is essential to establish a performance guideline to evaluate all materials in the same way. As the first publication reporting antiviral efficacy for mobility application, this paper will serve as a reference for future antimicrobial material development both in academia and industry. The addition of the antimicrobials showed changes in the mechanical properties of the TPE compounds. There was reduced tensile strength for all formulations, ZnPT and DOT combinations decreased by around 14%, 21% and 27%. Elongation at break was reduced by around 7%, 9% and 12% in the ZnPT and DOT combinations. The ZnPT and NaB formulation showed an increase in 100% Modulus (1.6–1.9 MPa) and 300% Modulus (2.6–2.9 MPa) compared to the control. TPE incorporated with ZnPT and DOT showed a 99.9% reduction against E. coli, S. aureus, A. albicans, A. niger, and, after heat aging testing, against Bovine Coronavirus, Adenovirus, Poliovirus, and Murine norovirus, with a 60-min contact time. This TPE formulation was molded into a handlebar grip for a scooter application. Details of the manufacturing and characterization of the TPE compound for handlebar grip application will be discussed in a future publication.

### CRediT authorship contribution statement

Zeynep Iyigundogdu: Conceptualization, Methodology, Writing – original draft. Basak Basar: Data curation, Investigation. Rachel Couvreur: Writing – original draft. Sandeep Tamrakar: Visualization, Investigation. Jaewon Yoon: Writing – original draft. Osman G. Ersoy: Investigation, Resources, Data curation. Fikrettin Sahin: Writing – review & editing, Resources, Data curation. Deborah Mielewski: Supervision. Alper Kiziltas: Writing – review & editing, Project administration.

### Table 10

| Contact time | BCoV | AdV | PoV | MNV |
|--------------|------|-----|-----|-----|
| Sample 1     | 3.33 | 2.97| 2.80| 2.93|
| Sample 5     | 3.08 | 2.91| 2.94| 2.83|
| Sample 6     | 2.80 | 2.94| 3.01| 2.94|

| % reduction  | BCoV | AdV | PoV | MNV |
|--------------|------|-----|-----|-----|
| Sample 1     | 99.95| 99.89| 99.84| 99.88|
| Sample 5     | 99.91| 99.87| 99.84| 99.85|
| Sample 6     | 99.84| 99.89| 99.90| 99.89|

### Table 11

| Sample no | Contact time | Log reduction | % reduction |
|-----------|--------------|---------------|-------------|
|           | 60 min       | 3.33          | 99.95       |
|           | 30 min       | 1.66          | 97.81       |
| Sample 1  | 10 min       | 1             | 90          |
|           | 5 min        | 1             | 90          |
|           | 1 min        | N/A           | N/A         |

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Declaration of competing interest

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

Data availability

Data will be made available on request.

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