J Polym Environ
DOI 10.1007/s10924-016-0924-y

ORIGINAL PAPER

Antibacterial and Drug Elution Performance of Thermoplastic Blends

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Abstract Food preservatives or drug compounds can be eluted from polymer substrates to prevent the occurrence of hospital-acquired infections and food spoilage. We investigated the antimicrobial and drug-elution properties of the albumin and zein thermoplastic blends plasticized with glycerol and mixed with varying amounts of low-density polyethylene (LDPE), food preservatives (sodium benzoate or sodium nitrite), and drugs (ampicillin or ciprofloxacin). *Bacillus subtilis* and *Escherichia coli* were utilized as Gram (+) and Gram (−) species, respectively, for antimicrobial and drug-elution analyses, since these species are common in the human body and in food environments. The amount of contamination occurring in food and medical applications could be limited with usage of plastic blends made from thermomechanical molding of proteins (albumin from hen egg white and zein from corn), drug eluting compounds, and low-density polyethylene.

Keywords Thermoplastic blends · Antibacterial · Drug elution · Food packaging · Medical plastic

Electronic supplementary material The online version of this article (doi:10.1007/s10924-016-0924-y) contains supplementary material, which is available to authorized users.

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Introduction

In medical and food packaging applications, the continued use of conventional plastic materials, such as polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET) have many drawbacks. These petroleum-based plastics lack an inherent property of preventing the growth of bacteria when contaminated, causing potential harm to individuals. Numerous strains of bacteria, such as *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*, have been found to be viable on the surface of plastics for over a month [1]. In a hospital environment, this bacteria contamination can lead to the further contamination of other surfaces, leading to potential cross-contamination [2]. In food packaging applications, foods may potentially spoil more rapidly when packaged with traditional plastics in comparison to food products packaged in a more sterile environment. In one study the cultures of *Lactobacillius* species and *Brocothrix thermosphacta* bacteria were found to be associated with the spoilage of refrigerated beef and pork which have been previously sterilized and placed in a vacuum-sealed plastic package after 30 days of refrigeration at 4 °C [3]. Another issue with the usage of conventional plastic materials in both medical applications and food packaging is the gradual leeching of chemicals from the plastic into the material contained within the plastic. In health care settings, compounds such as Bisphenol A and phthalates are able to leech into the body through transfusion or dialysis [4], while in food packaging it has been found that milk contained in bottles made from low density polyethylene (LDPE) is contaminated with naphthalene (utilized as a dispersant during plastic production) that gradually leeched from the plastic itself [5].

Multiple approaches have been studied to address the issue of bacterial contamination and growth in medical

and food packaging plastics. One approach involves the incorporation of additives in the conventional plastics that will lend antibacterial properties to the resulting plastic. In medical plastics, compounds such as sodium ampicillin [6] and ciprofloxacin [7] can be incorporated into the polymer substrate of utilized raw materials. For food packaging materials, it is possible to incorporate common food preservatives, such as sodium benzoate and sodium nitrite, [8] into the plastic that gradually leech into the food being contained. Surface treatments can also be utilized in the production of antimicrobial plastics, since research has shown that coated plastics with antibacterial compounds such as nisin [9] or a combination of lysozyme and silver nanoparticles [10] to result in a plastic possessing antibacterial properties. Another approach includes the modification of the plastic surface that will come into contact with the bacteria. In medical applications, the plastic surface can be lubricated to prevent the adhesion of bacteria when in contact [11], as well as nanotexturing of films with tetrahyrdofuran to generate a more hydrophobic surface when the film is treated with ethanol or methanol [12] to prevent bacterial adhesion. Hydrophobic surfaces can also be imparted onto food packaging films through the use of shrink-inducing to make a super-hydrophobic substrate, preventing bacteria from adhering to the surface [13].

To address the lack of antimicrobial properties in current conventional plastics, the use of alternative raw materials such as proteins in the production of plastics has been examined in this study. In particular of note are the proteins of albumin from the hen egg white and the zein protein from corn. With the use of plasticizers, it is possible to utilize both of these proteins in the production of plastics that could be utilized in the areas of food packaging and medical applications [14, 15]. One possible advantage of these alternative materials is their antimicrobial potential. In albumin-based bioplastics plasticized with glycerol, there is no promotion of the growth of bacteria (*E. coli* and *B. subtilis*) on the surface of the plastic [16]. Zein plastic films blended with antibacterial compounds such as lysozyme and a chelating agent disodium EDTA showed a decrease in bacterial growth as well as antioxidant activity [17]. When albumin and zein proteins were loaded with compounds such as ciprofloxacin hydrochloride, the proteins possess the similar elution properties that were present in conventional plastics, allowing for potential use in medical applications [18]. Albumin and zein-based plastics also possess the advantage of degradation, as they have been found to degrade in soil environments containing bacteria within ninety days [19]. A major drawback of using plastics based from pure proteins is their relative lack of mechanical properties when compared to a pure thermoplastic (such as LDPE), and that the addition of thermoplastic would aid in making a more effective plastic [20].

Our objective for this study was to evaluate the antibacterial and drug elution properties of albumin-glycerol and zein-glycerol bioplastics and thermoplastic blends for potential use in medical or food packaging applications. The examination of thermoplastic blends (as opposed to the pure protein-based samples tested in previous studies) will make it possible to determine the extent to which a traditional polymer (LDPE in this case) can be added to a thermoplastic without being a detriment to the antimicrobial properties of the resulting material.

Experimental

Materials

Albumin (purity ≥99%) was obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA); the zein purified protein was acquired from Acros Organics (New Jersey, USA); and the low density polyethylene (LDPE) powder (M_w ~ 25,000) (500 micron) was obtained from Alfa Aesar (Ward Hill, MA, USA). The glycerol used as a plasticizer was obtained from Sigma–Aldrich with a purity ≥99%. For antibacterial and drug elution analysis, following materials were purchased for testing: Bacto tryptic soy agar, tryptic soy broth, and Mueller–Hinton agar from Bectin, Dickinson and Company (Sparks, MD, USA); Dey-Engley neutralizing broth from Remel (Thermo Scientific, Suwanee, GA, USA); agar–agar solution that consisted of granulated Agar–Agar from EMD (Gibbstown, NJ, USA) and sodium chloride from Baker (Phillipsburg, NJ, USA); and phosphate buffered saline solution from HiMedia (Mumbai, India). The materials examined in the elution study were the following: sodium benzoate and sodium nitrite obtained from Carolina Biological Supply Company (Burlington, NC, USA); ampicillin (sodium salt) obtained from IBI Scientific (Peosta, IA, USA); and ciprofloxacin obtained from TCI (Tokyo, Japan). The bacterial species of *Bacillus subtilis* [Gram (+)] and *Escherichia coli* [Gram (−)] were graciously provided Dr. Jennifer Walker at the Department of Microbiology at the University of Georgia.

Preparation of Compression Molded Samples

The molding of thermoplastic blends was performed on a 24-ton bench-top press (Carver Model 3850, Wabash, IN, USA) with electrically-heated and water-cooled platens. Stainless steel molds were used to form dog bone-shaped thermoplastic blends for antibacterial analysis of plastic surface. To form the plastics, protein, and plasticizer were mixed manually in predetermined w/w ratios to be placed into the molds described in Table 1. The mixture of protein, polymer, and plasticizer was prepared in small batches of

Table 1 Composition of albumin or zein bioplastics/thermoplastic blends

Name of thermoplastic blend	Protein (%)	Plasticizer (Glycerol—%)	Polymer (LDPE—%)	Tests
LDPE	0	0	100	1,2
Alb-Gly	75 Albumin	25	0	1–3
Alb-5LDPE	71.25 Albumin	23.75	5	1,2
Alb-10LDPE	67.5 Albumin	22.5	10	1
Alb-20LDPE	60 Albumin	20	20	1
Zein-Gly	80 Zein	20	0	1,2
Zein-5LDPE	76 Zein	19	5	1,2
Zein-10LDPE	72 Zein	18	10	1
Zein-20LDPE	64 Zein	16	20	1

Tests conducted 1—surface antimicrobial, 2—drug/food preservative elution, 3—elution kinetics

varying masses based on density of materials for dog bone plastics (≤6 g for albumin and albumin-LDPE blends, and ≤4 g for zein, zein-LDPE blends, and LDPE since zein and LDPE is less dense compared to albumin), while the DMA flexbars (prepared with spacers) were made of 2 g of albumin, zein, LDPE, albumin-LDPE, and zein-LDPE plastics.

Subsequently, the mixture was filled into the flexbar or dog bone cavity of the stainless steel molds, with plungers placed on top of the molds to prevent the mixture from leaking. After covering with a plunger, the molds were then compressed for a 5-minute molding time at 120 °C, followed by a 10-min cooling period for the protein plastics. Samples were prepared under a pressure of at least 40 MPa, as a certain minimum amount of pressure must be applied in order to be able to mold a plastic [21]. After the samples were cooled for 10 min under pressure, the pressure was released and the samples were removed. To prepare the films for drug elution analysis, the samples were molded using the same process that was used to make DMA flexbars, except in this process it is necessary to not use spacers in order to make a thinner sample. In preparation of the films, it was necessary to blend the protein and drug/food preservative powders in order to ensure a consistent blend throughout the plastic. After the blending of protein and drug/food preservative, the plasticizer was added. When plastic molding was completed, the plastic samples were conditioned at 21.1 °C and 65% relative humidity for 24 h before characterization for antibacterial, drug elution, and elution kinetics testing.

Antibacterial Testing of Plastic's Surface

The antibacterial properties of the conditioned plastics were measured using the ASTM E 2180-01 standard test method, in which the aqueous based bacterial inoculum remains in close, uniform contact in a "pseudo-biofilm" state with the plastic blends. For each blend type, the Gram (+) specie *Bacillius subtilis* and the Gram (−) specie *Escherichia coli* were utilized as bacterial cells to determine the efficacy of bacterial growth on the plastic surfaces. Following the equilibration of standardized culture banks of $1–5 \times 10^8$ cells/mL determined through the use of dynamic light scattering analysis, 1 mL of the culture was applied to 100 mL of agar slurry for inoculation. After inoculation for one minute the slurry was then immediately applied to a 9 cm^2 area of the plastic blends that had been swabbed with phosphate-buffered saline to promote adhesion by reducing surface tension. After the appropriate time of application of cultured agar (within one hour for 0-h samples and at least 24h for 24-h samples after incubation at 37 °C), the agar was removed from the plastic surface through both sonication (1 min) and vortexing (1 min) the plastics in 30 mL of Dey-Engley neutralizing broth. The neutralizing broth containing the agar was diluted five times in a 10^{-1} dilution set, and then the dilutions were applied to tryptic soy agar plates, which were incubated for 24 h at 37 °C. After incubation for 24 h, the culture plates were counted for microbial growth and averaged to determine colony forming units (CFU)/mL. Samples were run in triplicate (n = 3) for each protein-plasticizer combination (including the polyethylene plastic control sample) in order to ensure precision.

Drug Elution and Zone of Inhibition Study

The potential of the plastics to elute antibiotics and food-preservatives to generate zones of bacterial inhibition was determined by the performance standards for antimicrobial disk susceptibility tests; eleventh edition (M02-A11) that has been developed by the Clinical and Laboratory Standards Institute in Wayne, PA [22]. The plastic blends were prepared with four levels of drug or food preservative (0, 5, 10, and 15%) using the sample procedure listed in Sect. Experimental, with dry drug added to the plastic blend before compression molding. After preparation, the samples were then cut into disk-sized plastics that were applied to the surface of Mueller–Hinton agar dishes that had been already inoculated with either Gram (+) specie *Bacillius subtilis* or the Gram (−) specie *Escherichia coli* at a concentration of $1–5 \times 10^8$ cells/mL. After application, the plates were then incubated for 5 days at 30 °C, during which the zones of inhibition were measured every 24 h to determine the change of diameter of the inhibition zone size over time. Samples were run in triplicate (n = 3) for each plastic type-additive combination (including the LDPE plastic control samples) in order to ensure precision.

Drug Elution Kinetics

The in vitro release of ampicillin and ciprofloxacin from the albumin bioplastics blended with varying levels of drug or food preservative (0, 5, 10, and 15%) into phosphate-buffered saline (PBS) was determined by the immersion of the thermoplastic blends into 25 ml of PBS in centrifuge tubes. The centrifuge tubes were then placed in a 37 °C shaking bath at shaking speed of 50 rpm for 5 days. At 24 h intervals, the absorption of both ampicillin and ciprofloxacin was determined by a UV–VIS spectrophotometer (Shimadzu UV-2401 PC UV–Vis Recording Spectrophotometer) at the absorbance peaks of 230 nm [6] for ampicillin and 275 nm for ciprofloxacin [18, 23]. In order to determine concentrations of solutions, linear calibration curves were obtained by measuring the absorption of ampicillin and ciprofloxacin solutions at known concentrations as shown in Fig. 1. For ampicillin, the equation derived from the linear fit is $y = 0.07912x + 0.08022$;while for ciprofloxacin it is$y = 0.63685x + 1.20162$, where x is equivalent to the absorption measured at the specific wavelength, and y is equal to the concentration of drug in solution.

Statistical Analysis of Drug Elution Testing

Statistical analyses were performed by fitting a regression model to compare the ability of plastics to elute drug and to determine the effect of the addition of LDPE into plastics. For plastics containing 15% of the elution material, inhibition zones after 5 days were analyzed by fitting a two-way ANOVA using the statistical software of SAS and R. Box-Cox transformations were used to determine the appropriate transformations needed to satisfy the normality assumptions of the experimental errors.

Results and Discussion

Antibacterial Properties of Albumin and Zein Plastic Blends

Surface Antibacterial Testing

Surface antibacterial testing is conducted in order to determine if albumin or zein-based plastics have efficacy to prevent bacterial growth. Figure 2 shows that, after the application of inoculated agar to the surface of both albumin-based and zein-based plastics, the inhibitive effect of the plastic on surface bacteria growth decreases as the amount of LDPE in the thermoplastic blend increases. When comparing the antibacterial efficacy of plastics containing varying levels of LDPE, with the plastics that contain 20% of LDPE there remains at least 150 CFUs/mL after the application of Gram+bacteria, while with 5% of LDPE there are less than 25 CFUs/mL recoverable. Albumin-glycerol and zein-glycerol bioplastics are able to prevent the growth of bacteria on its surface after 24 h of application for both Gram+ and Gram− bacteria, due to potential glycerol leeching and antibacterial properties of the albumin and zein proteins itself [16, 24]. When we increase the LDPE (no antimicrobial efficacy) content to the thermoplastic blend, complete surface bacterial growth prevention on the resulting thermoplastic blend is not present. In the albumin plastics that contain 20% LDPE there is a 15.88% decrease in Gram+bacterial colonies, and for zein that contains the same amount of LPDE there is a 25.23% decrease. When there is only 5% of LDPE in the plastics, there is a 72.79% decrease in Gram+bacterial colonies for albumin plastics, while for zein plastics there is a 96.45% decrease. Zein/LDPE of 90/10 blend still

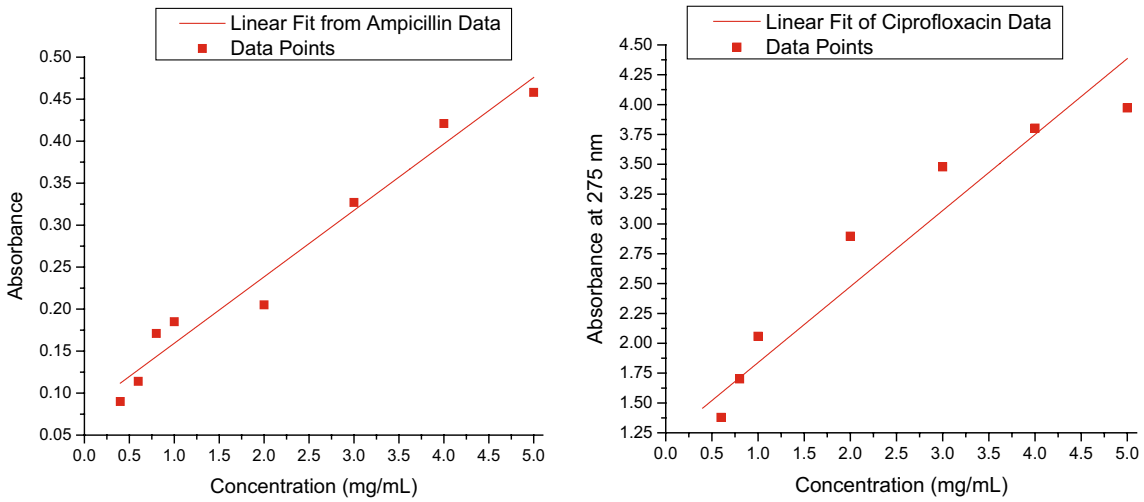

Fig. 1 Calibration curves of ampicillin and ciprofloxacin

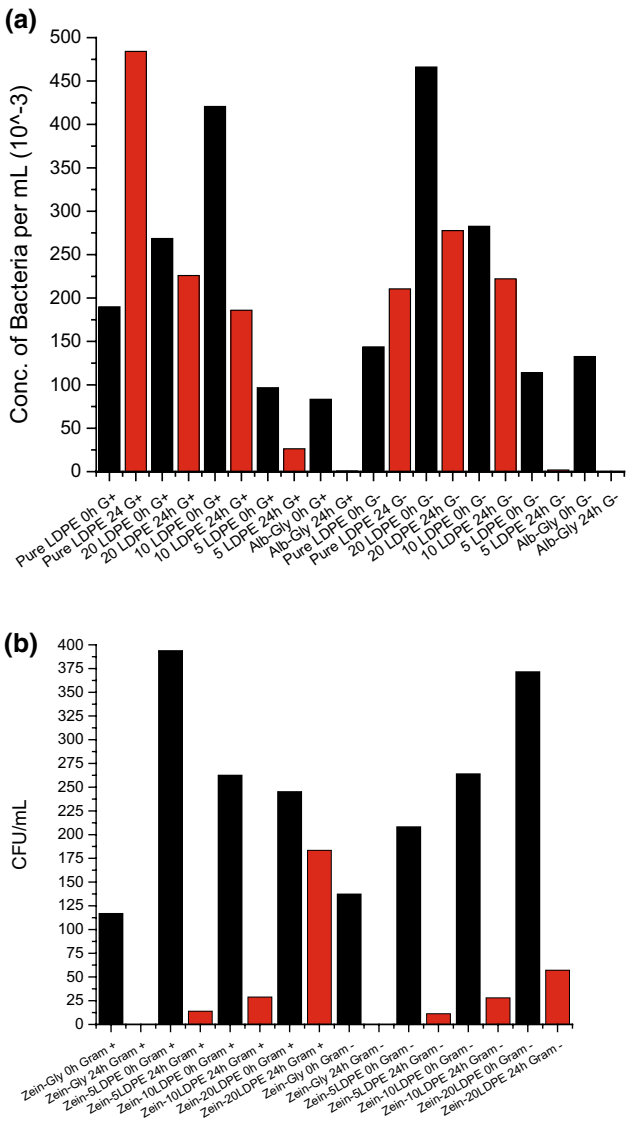

Fig. 2 Surface antimicrobial properties of **a** albumin plastic blends and **b** zein plastic blends

shows ~90% reduction in bacterial count after 24 h. This may be due to inherent hydrophobic and antimicrobial properties of zein protein.

When comparing the performance of albumin and zein plastics, we find that zein plastic antimicrobial effectiveness is not lessened as much by the addition of LDPE when compared to albumin plastics. For gram positive resistance, albumin plastics that consist of at least 10% LDPE (178 CFUs/mL) or 20% (224 CFUs/mL) will have higher bacterial growth after 24 h when compared to zein plastics (27 CFUs/mL for 10% LDPE, 184 CFUs/mL for 20% LDPE). This difference is also witnessed for gram negative bacteria testing, as the albumin plastics that contain 10% LDPE (221 CFUs/mL) or 20% LDPE (277 CFUs/mL) possess a lower microbial resistance when compared to zein-based plastics (28 CFUs/mL for 10% LDPE, 54 CFUs/mL for 20% LDPE plastics).

Our results corroborate with results found in past research on this subject, as plastics that have been incorporated with antibacterial additives such as nisin in PE-PEO films (84.6% inhibition after 3 days) [25] and chitosan-PEO films (3 $\log_{10}$ reduction after 24 h) [26, 27] as complete resistance to bacterial growth on plastic surfaces of thermoplastic blends is not possible without the use of additives specifically designed to prevent bacterial growth [8].

Drug Elution Properties of Albumin and Zein Plastic Blends

Additional antimicrobial properties need to be imparted into albumin and zein-based plastics for their use in medical and food packaging applications. To enhance antimicrobial properties, we have utilized two common medical drugs (ampicillin and ciprofloxacin) and two food preservatives (sodium benzoate and sodium nitrite) in the preparation of drug eluting plastics. Drug elution could make the prevention of bacteria growth in a given area possible, as opposed to the prevention of surface bacterial adhesion.

After imparting additional antibacterial properties into the thermoplastic blend through the elution of additives, we find that sodium nitrite is an ineffective additive to utilize, as the plastics in which it is imbedded do not generate any zones of inhibition on inoculated petri dishes, as shown in Figs. 3, 4, 5 and 6. The lack of effective antibacterial elution properties of sodium nitrite could be due to a lack of oxygen intake in the Petri dishes that allows anaerobic species to continue growth. Bacterial organisms are unable to absorb the sodium nitrite in an environment with low level of oxygen [28]. This lack of the inhibition zone may also be due to the potential lack of elution during the allotted time period. Sodium benzoate, on the other hand, demonstrates a gradual increase in the zone of inhibition over time, a sign of the release of benzoic acid into the agar. Benzoic acid will be generated by the dissociation of the sodium benzoate by the bacteria, releasing sodium hydroxide as well [29]. During the dissociation of sodium benzoate, the release of benzoic acid will reduce the pH of intracellular water by over 1 pH unit [30], inhibiting cell growth.

As shown in Figs. 7 and 8, we find that antibiotics ampicillin and ciprofloxacin are much more effective in inhibiting bacterial growth after 5 days for both Gram+ (43.4–39.2 mm for ampicillin, 42.1–37.7 mm for ciprofloxacin) and Gram− bacteria (35.2–19.4 mm for ampicillin, 38.5–41.7 mm for ciprofloxacin) when compared to sodium benzoate (15.2–8.1 mm for Gram+, 20.1–7.4 mm for Gram−) as shown in Fig. 9 and sodium nitrite (0 mm of inhibition for both bacteria; not shown). The inhibition zones of both plastics containing antibiotics increase

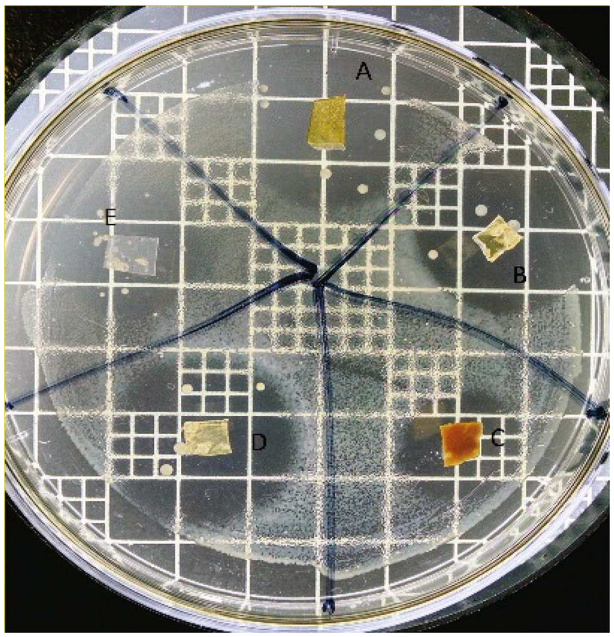

Fig. 3 Drug elution for Gram + samples. **a** zein-5LDPE-ciprofloxacin, **b** Alb-5LDPE-sodium benzoate, **c** zein-Gly-ampicillin, **d** Alb-Gly-sodium benzoate, **e** LDPE-ciprofloxacin

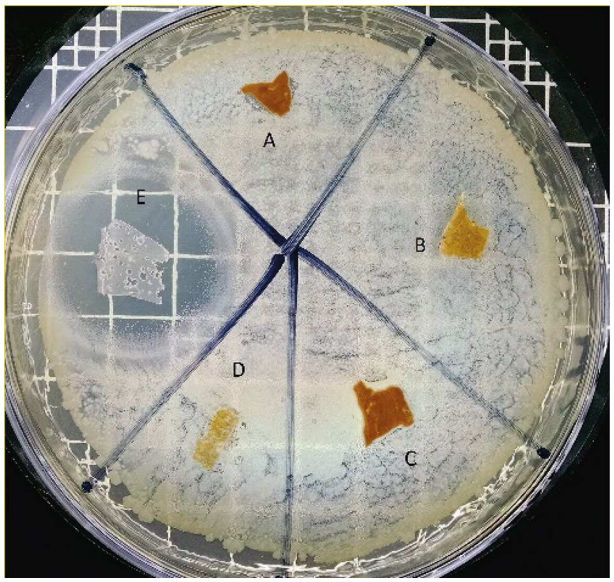

Fig. 4 Drug elution for Gram + samples. **a** zein-5LDPE, **b** Alb-5LDPE-sodium nitrite, **c** zein-Gly, **d** Alb-Gly-sodium nitrite, **e** LDPE-ampicillin

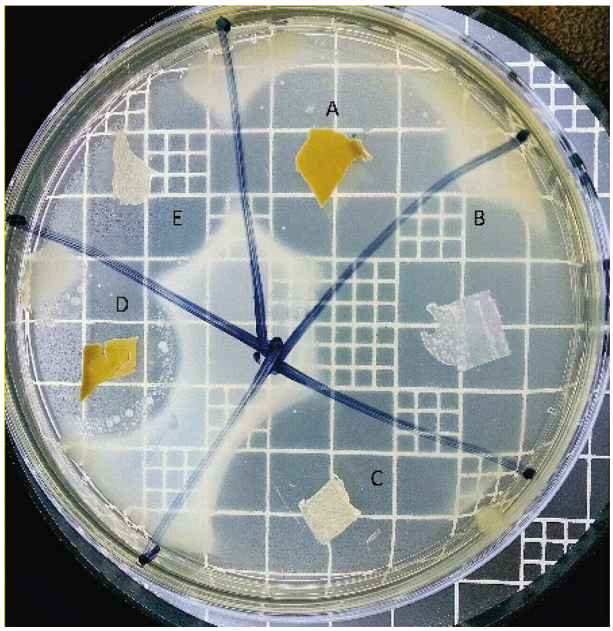

Fig. 5 Drug elution for Gram− samples **a** zein-5LDPE-ampicillin, **b** LDPE-ciprofloxacin, **c** Alb-Gly-ampicillin, **d** zein-Gly-sodium benzoate, **e** Alb-5LDPE-ampicillin

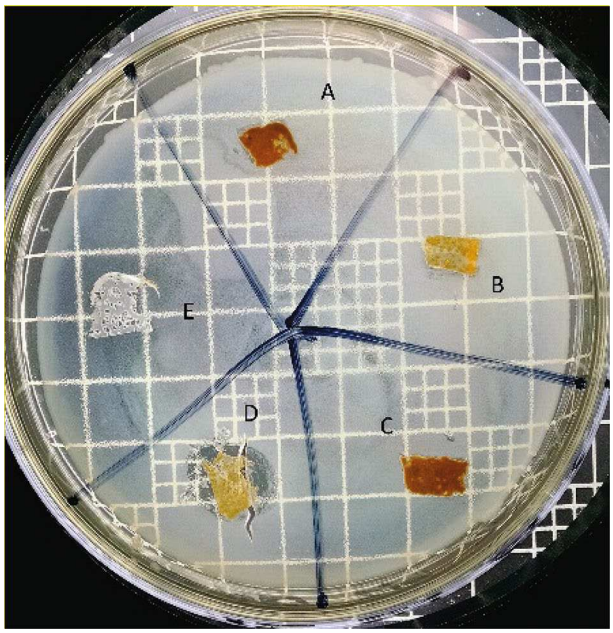

Fig. 6 Drug elution for Gram−samples **a** zein-5LDPE-sodium nitrite, **b** Alb-5LDPE, **c** zein-Gly, **d** Alb-Gly-sodium nitrite, **e** LDPE-sodium benzoate

in size as time passes. The inhibition of growth shows a linear trend for plastics containing ciprofloxacin over five days. Ciprofloxacin possesses the ability to inhibit both Gram+ and Gram− growth, as it has been designed to be effective against a wide range of bacterial organisms, and the ability to elute from a material easily [31]. While ampicillin possesses the ability to consistently inhibit Gram+ bacteria growth, for Gram− bacteria, the zone of inhibition stays at a consistent size (37.2–18.3 mm) over 5 days. Ampicillin lacks the same antibacterial effectiveness against *E. coli* when compared to ciprofloxacin

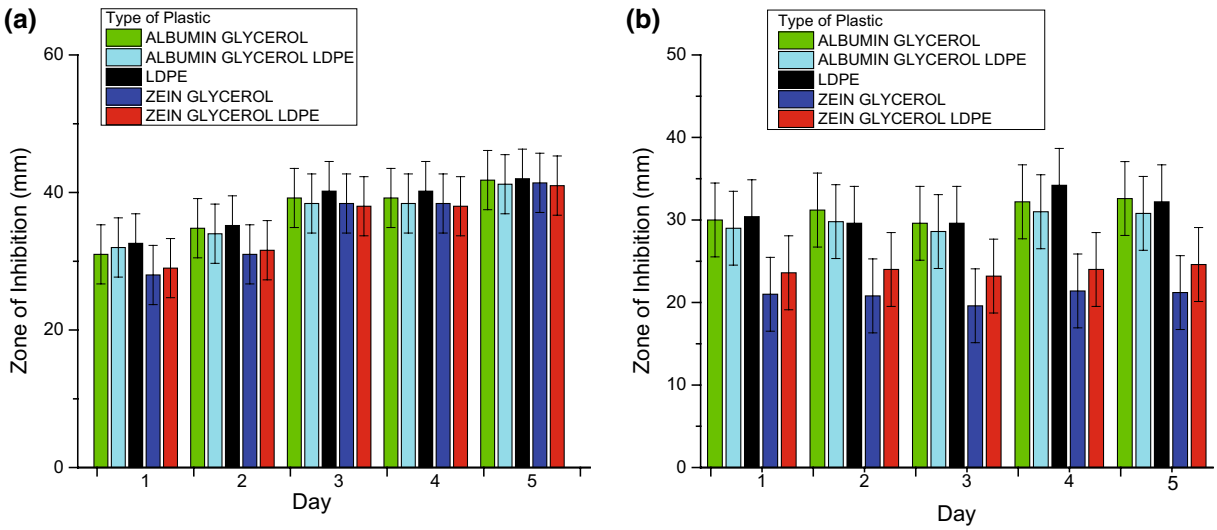

Fig. 7 Zone of inhibition for plastics with 15% of ampicillin: **a** Gram + and **b** Gram−

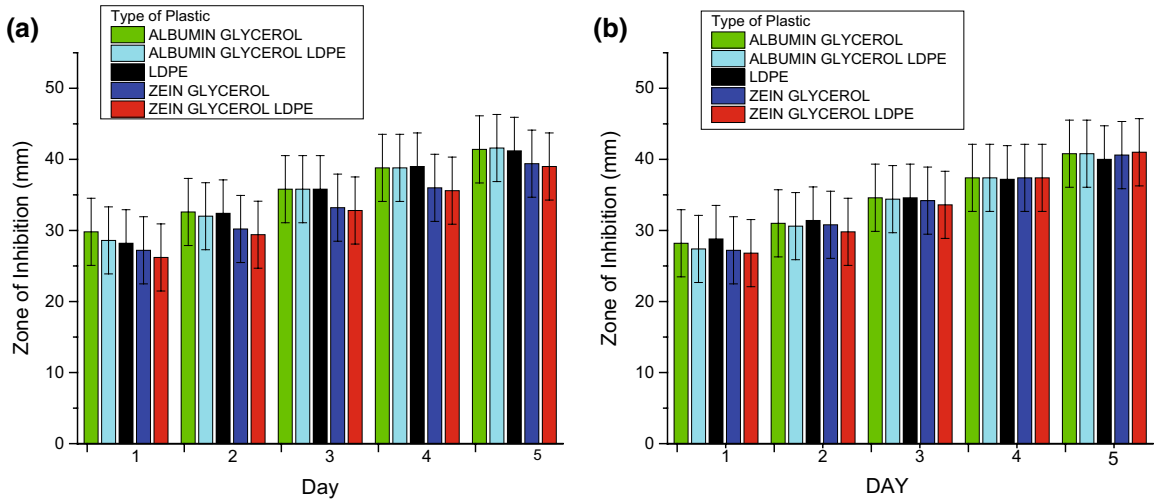

Fig. 8 Zone of inhibition for plastics with 15% of ciprofloxacin: **a** Gram + and **b** Gram−

because the bacteria are potentially gaining a resistance to the ampicillin [32].

Effect of Drug Concentration on Zone of Inhibition

To determine the effect of drug/food preservative levels on the inhibition zones generated by plastics, we loaded 5 and 10% additives into the plastics. The results are compiled in Figs. 10, 11 and 12. When we modify the plastics to contain lesser amounts of the antibiotics, we find the overall size of the inhibition zones will decrease, as well as an increase of the variability of inhibition zone size. The decrease in inhibition zone size is caused by lower amounts of antibiotic released from the plastic, with the potential formation of drug resistance by the bacteria if the dose of antibiotic in the environment is too low. We also find that the results of plastics containing 10 and 5% of loaded drug possess a higher degree of variability when compared to plastics containing 15% of loaded drug. Since there is less antibiotic in the plastic, there is an increase in probability that the drug release from the plastics will not be as uniform, which increases variability [33]. Albumin-based plastics tend to have relatively higher zones of inhibition when compared to the pure LDPE and the zein plastics, with increased levels of drug elution possible. Albumin-based plastics may demonstrate an increased ability to elute drugs and food preservatives in comparison to zein and LDPE plastics;

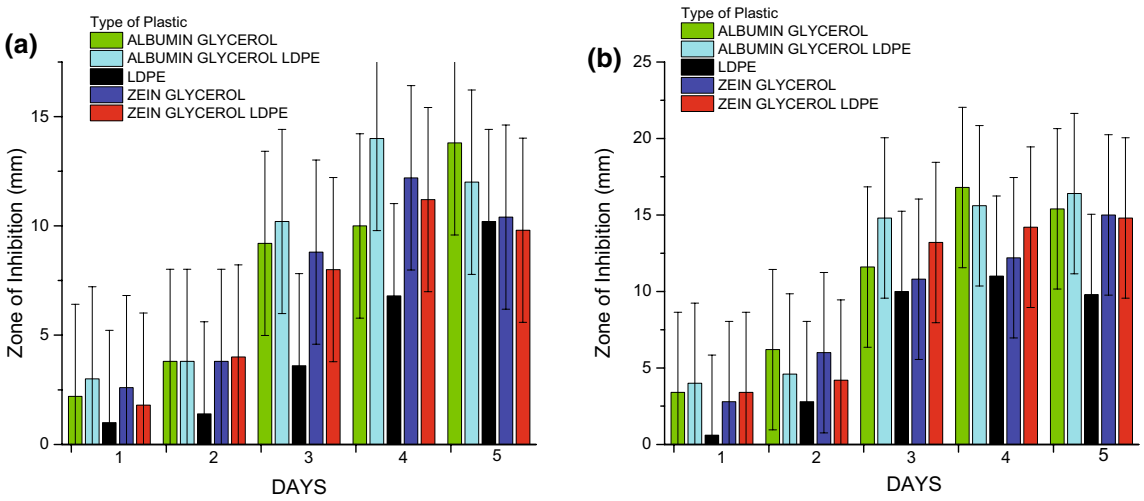

Fig. 9 Zone of inhibition for plastics with 15% of sodium benzoate: **a** Gram + and **b** Gram−

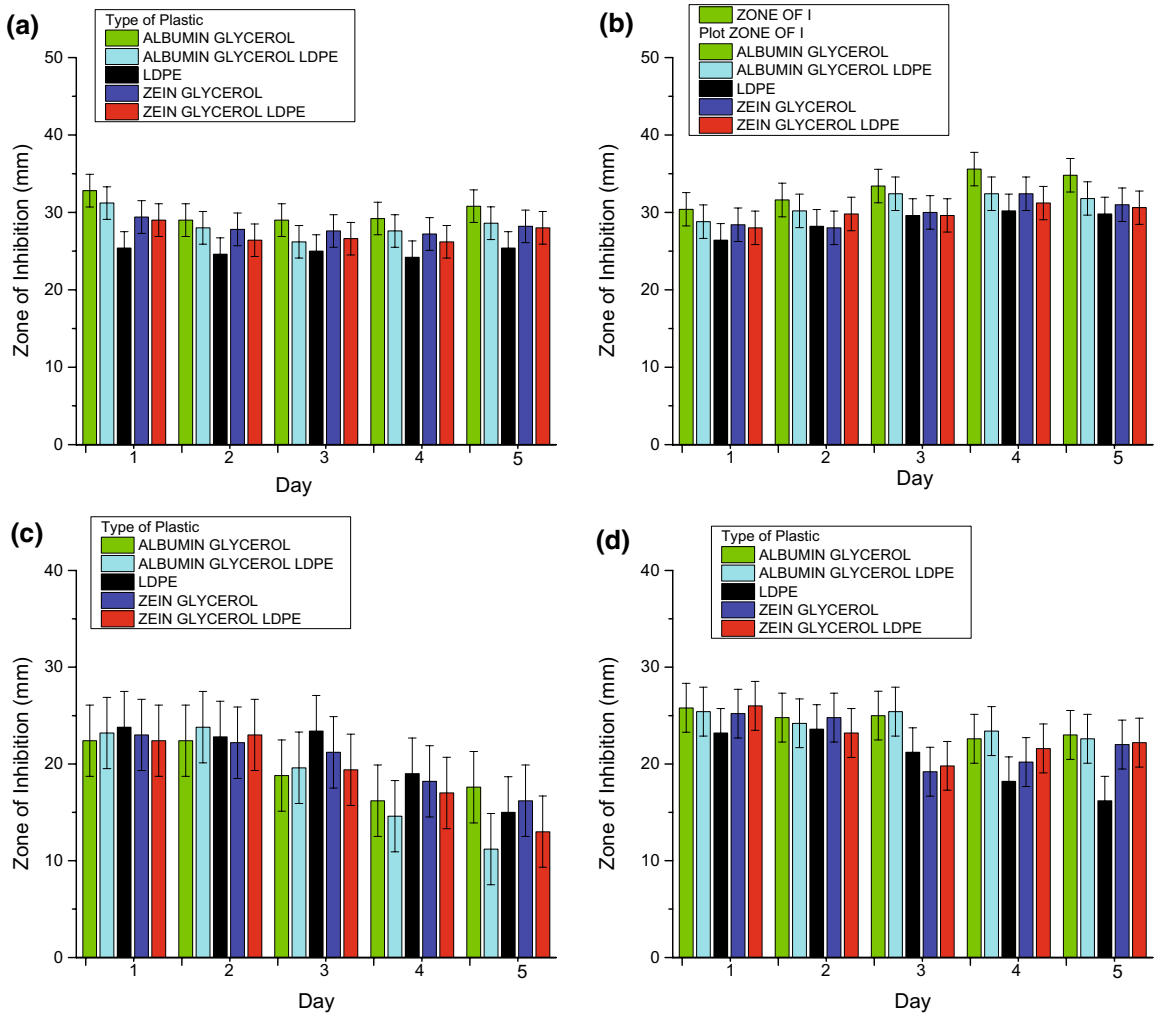

Fig. 10 Zone of inhibition for plastics with ciprofloxacin: 10%—**a** Gram + and **b** Gram−; and 5% **c** Gram + and **d** Gram−

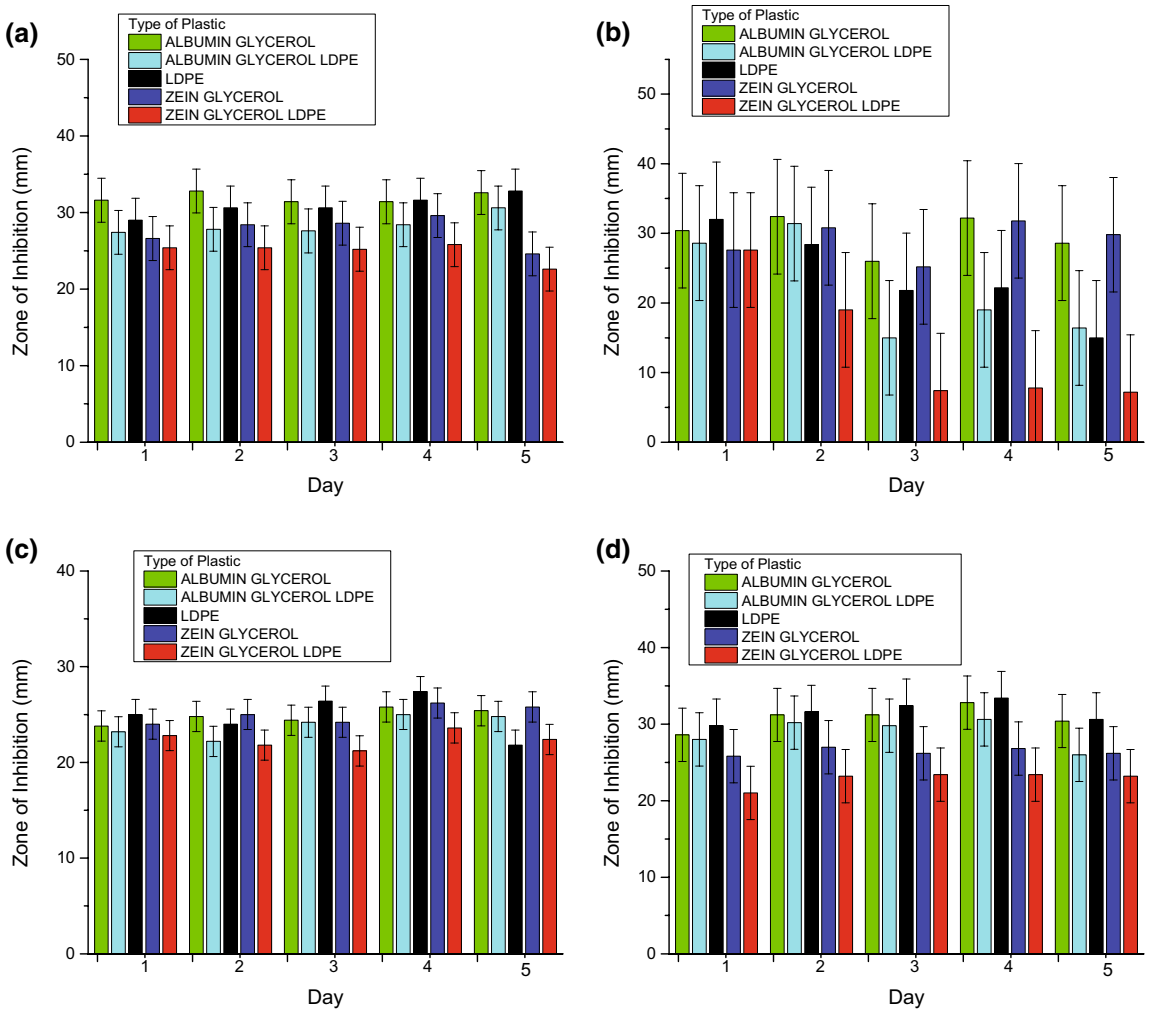

Fig. 11 Zone of inhibition for plastics with ampicillin: 10%—**a** Gram + and **b** Gram− ; and 5% **c** Gram + and **d** Gram−

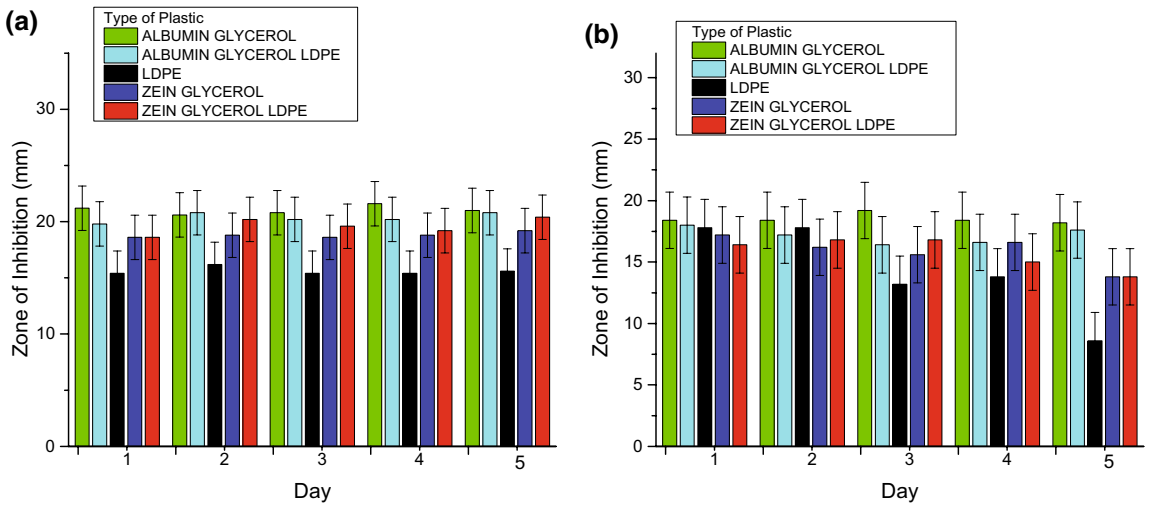

Fig. 12 Zone of inhibition for plastics with **a** 10% and **b** 5% of sodium benzoate: Gram−

albumin is more permeable in areas that contain higher moisture such as bacterial colonies [34].

For the sodium benzoate plastics, when we decrease the amount of food preservative in the plastic, the plastics will be unable to produce a zone of inhibition when encountered with a Gram+ bacteria. This lack of effectiveness against Gram+ such as *B. subtilis* may be due to sodium benzoate's inability to generate enough benzoic acid in solution to eliminate Gram+ colonies at lower concentrations [35]. We also find that much like the plastics that have been loaded with antibiotics, the sodium benzoate containing plastics will have a much higher level of variability in the zone of inhibition generated when encountering Gram− species, which could due to the lack of even dispersion in the plastic.

Statistical Analysis of Drug Elution on Zone Inhibition (15% drug w/w)

Inhibition Zone Analysis for Albumin Bioplastics and Zein Bioplastics

Certain inferences can be made from the statistical analysis of the drug elution experimental raw data. To perform this analysis, we develop a regression model with the diameter of the inhibition zone as the response and types of proteins and drugs or preservatives as explanatory variables. One standard assumption for fitting a regression model is that the errors are identically and independently distributed Normal random variables with zero mean and some constant variance. However, this assumption will not be valid since there is (almost) no inhibition for the control (no drug) and preservative, sodium nitrite. As seen in Fig. 13, from the boxplots that compare the resulting inhibition for different drugs and food preservatives, we conclude that we should concentrate on sodium benzoate, ampicillin and ciprofloxacin only.

After the elimination of sodium nitrite as a potential additive, we can now fit a regression model with the diameter of the inhibition zone as the response and different types of proteins and three drugs/food preservatives (sodium benzoate, ampicillin and ciprofloxacin) as explanatory variables. We entertain both main effects of proteins and drugs as well as the interactions between proteins and drugs in our model, and fit two separate models for Gram+ and Gram− bacteria. As shown in the Supplemental information (Appendix 1), for both regression models, we determine that the factors of proteins and drugs/food preservatives are both statistically significant, as well as the interaction between the proteins and drugs/food preservatives, for both Gram+ and Gram− bacteria. When comparing the influences of the drugs and proteins on expected results, the sum of squares corresponding to the factor of drugs is 14,652 out of a total of 15,729, while the factor Gram− bacteria it is 7684 out of the total of 9739, indicating the weight of these factors in the amount of variation that can be seen in the data. As the type of drug/preservative incorporated explains most of the variation in data, we determine that the use of protein has the greatest influence for both Gram+ and Gram – bacteria.

With the examination of the regression coefficients for both Gram+ and Gram− negative data, many inferences can be made. For the Gram+ bacteria, we find that the combination of albumin and ampicillin results in the maximum amount of predicted inhibition (3.6 + 34.8 + 6.4−4.2 = 40.6 mm), followed by zein and ciprofloxacin (3.6 + 35.8 + 6

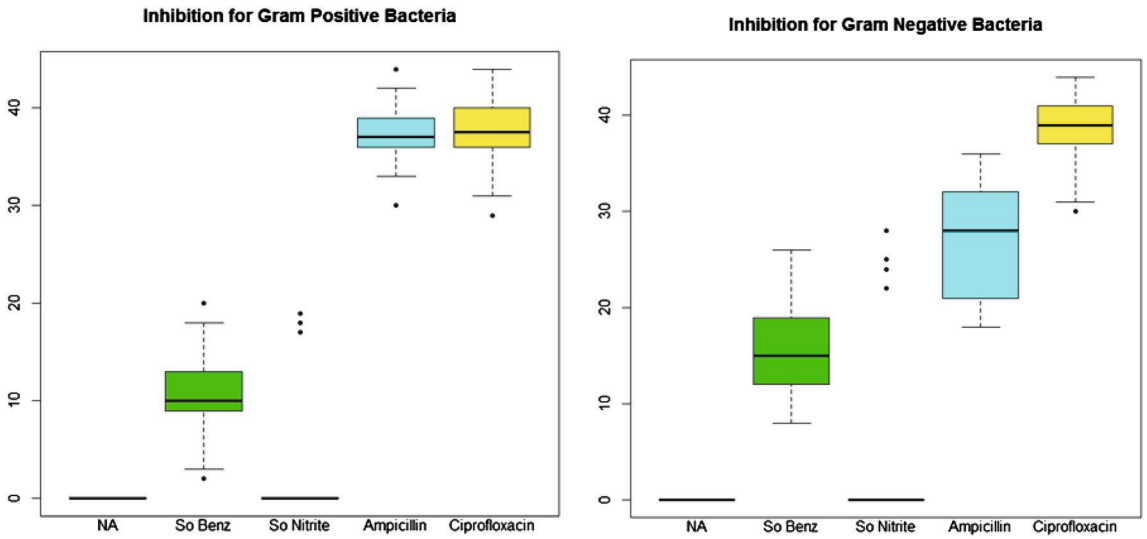

Fig. 13 Boxplot of the inhibition zones to compare drug/food preservatives, where drug level is equal to 15% w/w of total plastic

.8−6.0 = 40.2 mm). Albumin and LDPE samples that contain ciprofloxacin are also good, with predicted inhibition being 39.6 and 39.4, respectively. As for the Gram– bacteria, we find that the combination of albumin and ciprofloxacin would result in the maximum amount of predicted inhibition (9.8 + 29.6 + 5.6–2.4 = 42.6 mm), then followed by zein and ciprofloxacin (9.8 + 29.6 + 5.6–4.8 = 40.2 mm) and LDPE and ciprofloxacin (9.8 + 29.6 = 39.4 mm).

When we compare the individual types of protein/polymer as well as the type of additive utilized, several findings are determined. Through the use of our regression model, we find that that the additive of ciprofloxacin is best for the prevention of Gram− bacteria growth, as it will generate the largest inhibition zones when compared to the other three drugs/food preservatives. As for the type of plastic sample, zone of inhibitions will be greatest when albumin is utilized as the material, with zein in a close second, and LDPE with the lowest inhibition zones. When we conduct the same type of regression analysis for the Gram + results, we find that both the additives of ampicillin and ciprofloxacin are highly effective in the prevention of bacterial growth. The regression model suggests that the combination of albumin with ampicillin as an additive will lead to the largest zone of inhibition, with any of the plastic types (albumin, zein, or LDPE) being effective in bacterial growth prevention when blended with ciprofloxacin.

Inhibition Zone Analysis for Albumin and Zein thermoplastic blends

It is necessary to then examine the effect of the addition of LDPE into the plastic blends on the level of drug elution (the size of inhibition zone produced), as this will determine if the material will still be suitable for elution applications. Since we determine that the interaction is significant, it will be appropriate to consider each protein separately. However, when we compare both albumin with albumin blended with LDPE and zein and zein blended with LDPE, let us consider models without interaction and fit the model to the data points pertaining to either albumin and albumin-LDPE or zein and zein-LDPE. This lack of interaction makes it possible to determine the antimicrobial efficacy of a material based on the level of LDPE contained in the plastic without being affected by the type of protein in the plastic. In the comparison between albumin and albumin-LDPE, we see that there is no significant statistical difference in the size of the inhibition zone generated between the two materials for both Gram+ and Gram− bacteria; the p-values in the ANOVA tables are shown in the Supporting information (Appendix 1). For the zein and zein-LDPE comparison, the same inferences can be made for both Gram+ and Gram−, as shown in the ANOVA tables in Tables 2, 3, 4 and 5. However, given the p-value corresponding to the proteins for Gram + bacteria, we can conclude that adding LDPE does not make any different at a 10% level of significance. These conclusions are based on a model containing the additives of sodium benzoate, ampicillin and ciprofloxacin, but the conclusions and will essentially not change even if the control (no drugs) and sodium nitrates are included in the model.

Elution Kinetics of Albumin Bioplastics

Since the albumin-based bioplastics that contained ampicillin and ciprofloxacin possess the greatest ability to generate inhibition zones, we examine further the elution kinetics of these samples. We analyze the kinetics of drug elution for albumin-glycerol bioplastics, containing ampicillin and ciprofloxacin at 5, 10, and 15% concentrations, using the formulations we have previously utilized. When analyzing the albumin bioplastics that contain either drug, we find that the amount of drug loaded into the plastic is crucial to the amount of antibiotic that will be released over a given period of time, as illustrated in Fig. 14. With the albumin that contains 15% of ampicillin, we find that it will elute more ampicillin in solution in 1 day than the 5% ampicillin-containing samples will elute in 5 days, or the 10% ampicillin-containing samples will elute after 3 days. Albumin bioplastics that contain 15% of ampicillin can elute more drug due to the fact that they contain more drug, as this allows more ampicillin to be released over time after its initial release [36]. For the albumin bioplastics containing ciprofloxacin, the release of drug from the plastic is more gradual. The plastic that contains 15% ciprofloxacin will release a considerably higher amount of antibiotic after 5 days in solution when compared to albumin plastics containing 10 and 5% of ciprofloxacin. Based on the time required to release ciprofloxacin from albumin bioplastics (there was little difference in all of the drug levels before 5 days of analysis), ciprofloxacin may be bound to the albumin-glycerol material in a way that inhibits an immediate release when compared to other drugs [37].

Table 2 ANOVA table for examining influence on zone of inhibition when LDPE (5, 10, and 20% w/w) is added to albumin protein and drug (15% w/w) during plastic production for Gram+ Bacteria

	Df	Sum Sq	Mean Sq	F value	Pr(> F)
Factor (Protein)	1	11	10.8	2.368	0.136
Factor (Drug)	2	5116	2558.0	560.783	<2e-16
Residuals	26	119	4.6		

Table 3 ANOVA table for examining influence on zone of inhibition when LDPE (5, 10, and 20% w/w) is added to albumin protein and drug (15% w/w) during plastic production for Gram − Bacteria

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Factor (Protein)	1	17.6	17.6	2.01	0.168
Factor (Drug)	2	3071.7	1535.8	175.09	8.21e-16
Residuals	26	228.1	8.8		

Table 4 ANOVA table for examining influence of LDPE on zone of inhibition when added (5, 10, and 20% w/w) is added to zein protein and drug (15% w/w) during plastic production for Gram+ Bacteria

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Factor (Protein)	1	24	24.3	6.897	0.0143
Factor (Drug)	2	5086	2542.8	721.755	< 2e-16
Residuals	26	92	3.5		

Table 5 ANOVA table for examining influence of LDPE on zone of inhibition when added (5, 10, and 20% w/w) is added to zein protein and drug (15% w/w) during plastic production for Gram − Bacteria

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Factor (Protein)	1	0.8	0.8	0.059	0.81
Factor (Drug)	2	2933.6	1466.8	103.333	4.24e-13
Residuals	26	369.1	14.2		

Conclusions

When we compare the surface antimicrobial properties of the protein- thermoplastic blends, we find that adding more LDPE into the thermoplastic blend will diminish the antimicrobial properties that are witnessed in pure-protein bioplastics. However, the addition of food preservatives and drugs into the thermoplastic blends will have varying degrees of antimicrobial properties due to elution, as it was demonstrated that pure albumin-glycerol bioplastics loaded with the antibiotics of ampicillin or ciprofloxacin provide the best drug elution properties of all of the thermoplastic blends analyzed. In comparison, the use of no drugs or food preservatives were less effective in the prevention of bacterial growth on Petri dishes. Since these plastics would need to be examined for the potential use in medical applications, such as medical devices, in the future, it will be necessary to test these materials under methods such as ASTM F2097–10: Standard Guide for Design and Evaluation of Primary Flexible Packaging for Medical Products, or ASTM F813–07(2012): Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices. A more detailed analysis of how bacteria is inhibited by drug elution (through pH change, nutrient deprivation, etc.) will be necessary for expanded use beyond laboratory experiments for compounds such as sodium benzoate. The examination of the elution of the drug/food preservative of albumin or zein bioplastics over a longer period of time (7+ days) will also be necessary, as medical and food packaging will be placed in storage longer than a week before utilized. Also using other types of drugs, such as amoxicillin and moxifloxacin, as well as other types of bacteria, such as Staphylococcus aureus and Neisseria meningitidis, should be analyzed to obtain deeper understanding of drug/food preservative effectiveness in preventing bacterial growth of variety of species, depending on specific end use.

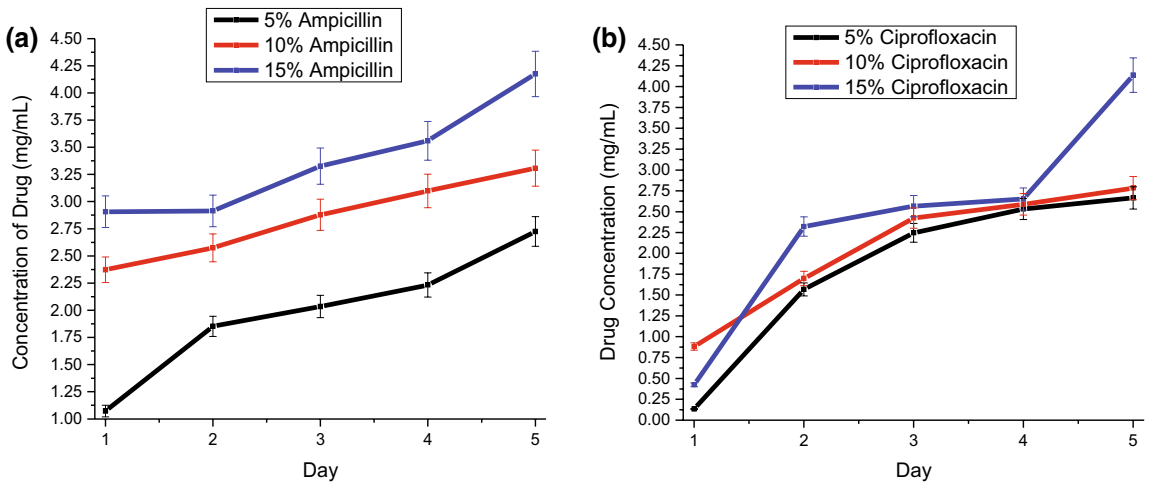

Fig. 14 Elution rate of drug from albumin-glycerol bioplastics: **a** ampicillin and **b** ciprofloxacin

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