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Biodegradable antimicrobial films based on poly(lactic acid) matrices and active azo compounds

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ABSTRACT: Using solvent casting and melt compounding methods, we realized antibacterial and antifungal poly(lactic acid)-based films by introducing different percentages of antimicrobial azo dyes into polymer matrices. Concentration up to 0.01% (w/w) of azo compounds permitted the preparation of antimicrobial and transparent films. The thin films retained the properties of the pure PLA matrices, such as glass transition temperature, flexibility, and amorphous nature. The films exhibited antimicrobial activity and the capability to inhibit biofilms formation of *Staphylococcus aureus* and *Candida albicans*. Spectrophotometric investigation of azo compounds release from the polymer matrices confirmed that the materials might have applications in fields where an intrinsic antimicrobial ability of the material is required, such as biomedical tools, biodegradable antibacterial coatings, and films for active packaging. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42357.

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INTRODUCTION

Infections by pathogenic microorganisms are of great concern for the realization of medical devices as well as food packaging materials.[1,2] The appearance of microorganisms resistant to the most common drugs increases this concern.

Bacterial infections are frequently associated with the use of medical devices, therefore it is essential to develop new materials with antimicrobial activity to solve the problem of contamination by microorganisms.[3,4] In an effort to address this problem, commercial antibacterial agents have been incorporated in numerous types of materials for medical devices.[5,6] Several antimicrobial agents have been introduced into polymers; the most common antimicrobial agents used in polymer films are triclosan, chlorhexidine, tetracycline and derivatives, benzophenon, and rifampicin.[1]

Our strategy is to insert new synthetic antimicrobial azo compounds into polymer materials with excellent biocompatibility.

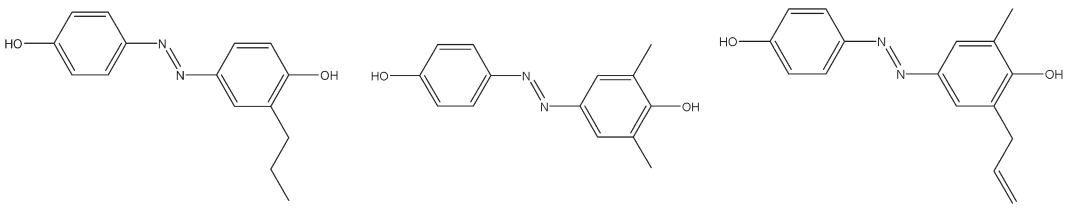
Recently we synthesized a new class of antimicrobial compounds with azobenzene structure.[7,8] These compounds showed a strong activity against Gram+ bacteria and fungi. The ones having the strongest bactericidal activity and the ability to inhibit the biofilm formation have already been selected for incorporation into commercial polyolefin matrices, in order to produce composites with low production cost, good processability, and antimicrobial potential.[9]

In this work we consider the insertion of three of these molecules (namely A3, A4, and A5) in biodegradable polymers, addressing chemical issues, microbiocidal activity, stability, and processability of the final material.

We focused our attention on poly(lactic acid) or polylactide (PLA) as a representative example of biodegradable polymers, suitable for the realization of thin films.[10–12]

PLA is a thermoplastic aliphatic polyester obtained by ring-opening polymerization of lactide derived from the fermentation of sugar feed stocks such as corn. Recent development of a continuous process of production of this resin has lowered the price of PLA to the point where it is now competitive with other degradable polymers and potentially competitive with petroleum derived plastics.[13] PLA has found applications in the medical and packaging fields because of its promising mechanical properties, its potential for biodegradability and biocompatibility.[14–18]

Additional Supporting Information may be found in the online version of this article.



Scheme 1. Chemical structures of azo compounds A3, A4, A5.

Its biocompatibility and bioresorbability has made it a suitable choice for applications such as drug delivery systems, sutures, or blood vessels.[19] Recent work has shown the efficacy of using electrospun PLA/silk–gelatin fibers as tubular scaffolds to support cell growth.[20]

The main drawbacks of PLA are related to its "slow" crystallization kinetics[19] compared to the most common polymers like polypropylene or polyethylene, the processing window limited by thermomechanical degradation, and last but not the least the deterioration of mechanical properties when heated.[11] To overcome some of these problems, in general, several additives to polymeric materials can be adopted: plasticizers, lubricants, pigments and coloring agents, expanders, nucleating agents.[21,22]

PLA has also been used for antimicrobial food packaging applications, mainly by incorporation of natural antimicrobials into polymer matrix.[23–26] The main disadvantage is the poor thermal stability of the active molecule. Most of the natural antimicrobial agents show low resistance to excessive heat treatments applied during processing. In all these cases the extrusion is not be suitable for the production of natural antimicrobial agent incorporated packaging materials, such as pectin/PLA formulations.

The choice of the system azo compound/PLA permitted the preparation of novel materials by two simple methods: solvent casting (SC) and melt compounding followed by compression molding (MC). The latter method is of particular interest, because it allows a scale-up to industrial production.

Using these two methods, we introduced different percentages of active azo compounds in different PLA matrices. The polymers were loaded with low percentages of A3, A4, and A5 azo compounds[7] (from 0.01% to 0.1%), reported in scheme 1.

We prepared transparent amorphous films, as confirmed by X-ray analysis. The films retained the thermal proprieties, flexibility and shine of the pure PLA matrices, without azo compounds.

The films exhibited biocide activity. In fact, preliminary tests of *Staphylococcus aureus* biofilm formation on PLA films were analyzed by staining with crystal violet, showing a complete discoloration at a concentration of at least 0.1% of azo compounds. Finally, we performed azo compounds release studies from the polymer matrices by spectrophotometric methods, in order to establish the stability of the final composite materials.

EXPERIMENTAL

Materials and Methods

All reagents and solvents were purchased from Sigma Aldrich and Carlo Erba and used without further purification.

The azo compounds A3, A4, and A5 were synthesized and purified according to our previous work (Piotto *et al*, 2013).[7]

The selected polymer matrices were PLA 4032D and PLA 4060D from NatureWorks® LLC. The average molecular weight (M_w) of both grades is in the range 190-200 KDa, whereas the polydispersity index was about in the range 1.7–1.9. The main difference among the two grades is the D-lactide content which is about 12% for 4060D and about 1.5% for 4032D. As a consequence, the 4060D grade is amorphous and the 4032D is semi-crystalline, with a maximum crystallinity degree of about 45%.[27]

Film Preparation

Melt Compounding and Compression Molding Method. PLA 4032D was dried in vacuum at 35°C for one week before the extrusion. This step is necessary to avoid the matrix degradation during the hot mixing.[28] We prepared three matrices containing 0.01%, 0.05%, and 0.1% of antimicrobial agents A3, A4, and A5 in PLA 4032D. We also extruded the polymer without the azo compound to use as blank. The dry materials were mixed by melt compounding in a counter-rotating twin-screw microcompounder (HAAKE MiniLab II Micro Compounder, by Thermo Scientific) with an integrated backflow channel. Using this device is possible to work with a total amount of only 5.0 g of material, being ideal for expensive or small scale materials such as drugs.

The Haake Minilab II was operated with "starve feeding". According to this method, weighed amounts of materials were added to the hopper of the extruder in order to control the percentage of the antimicrobial agent inside the polymer matrix. Because of the backflow channel and a bypass valve, the residence time is well defined, and the materials were mixed at 180°C for 5 min at 60 rpm. According to previous studies,[7,9,28] these conditions allow extrusion and minimization of PLA and azo dyes degradation. Blended materials were cooled and collected as a solid ribbon; they looked perfectly transparent and without bubble air inside. After a few days under vacuum, the dried matrices were placed between two sheets of sterile Teflon and molded by means of a Carver press at 180°C to obtain thin films of about 150 μm thickness. The film thickness was measured by a thickness gauge.

Solvent Casting Method. We have also realized two series of PLA films by solvent casting method: PLA 4060D and a mixture of PLA 4060D and PLA 4032D. We added Tween 80 as plasticizer[29] to improve the mechanical properties, and to avoid crystallization during the SC method. We could not prepare film formed by only PLA 4032D, because we observed crystallization during peeling step. Using a mixture of polymers we overcame this problem and obtained homogenous and amorphous films.

The total amount of the matrix formed by plasticizer and polymeric pellets was 0.5 g. In the first film series we used 0.45 g PLA 4060D as matrix and in the second one a mixture of 0.25 g PLA 4060D and 0.20 g PLA 4032D. In both cases we added 10% (w/w) of Tween 80 as plasticizer.

For the preparation of solvent-casted films, we dissolved weighted amount of polymeric matrix and Tween 80 in 10 mL of chloroform with vigorous mixing at room temperature for 5 h. Separately, we prepared three different stock solutions by dissolving 1 mg of A3, A4, and A5 azo compounds in 1 mL of acetone. We split the matrix in different beakers, adding in each the exact volume to reach the final concentration of 0.01%, 0.05%, and 0.1% (w/w) of antimicrobial agent. After gentle mixing at room temperature for a few minutes, the viscous solutions were poured onto different glass Petri dishes, and left for 24 h at room temperature. After solvent evaporation the films were peeled intact from the casting surface. Homogeneous and elastic films were obtained, approximately 700 μm thick, as measured by a thickness gauge.

Thermal, Mechanical, and Structural Characterization. Thermal measurements were performed by a DSC-7 Perkin Elmer calorimeter under nitrogen flow at 10°C/min rate. Polarized optical microscopy was performed by a Jenapol microscope fitted with a Linkam THMS 600 hot stage. Dynamic mechanical tests were carried out on film samples using a TA Instruments DMA 2980 analyzer. DMA spectra were recorded in the tensile mode at 25°C, with frequency in the range 1-100 Hz. X-ray diffraction patterns on film samples of the polymers and of powder sample of azo compounds were recorded on a flat film camera using Ni-filtered Cu-K_α radiation and a Philips X-ray generator, with a camera to sample distance of 9.0 cm. The Fujifilm MS 2025 imaging plate and a Fuji Bioimaging analyzer System, model BAS-1800, were used for digitizing the diffraction patterns. Wide-angle X-ray powder diffractions (WAXD) for a crystalline powder sample of azo compound and a PLA film sample were obtained with Ni-filtered Cu-K_α radiation by a Brucker D8 Advance diffractometer. For release studies, a Perkin Elmer Lambda 900 Spectrophotometer was used, working in time-drive mode, at a fixed wavelength of 358 or 357 nm.

The hydrophobic properties of the obtained samples were assessed by means of static water contact angle (CA) measurements, performed by depositing five drops of distilled water each of 2 μL on the film surface and then calculating the average values for each side. Experimental measurements were performed with a First Ten Angstrom Analyzer System 32.0 (mod. FTA 1000) according to the standard test method (REF Standard test method ISO15165-1).

Antimicrobial Characterization. We tested films with 0.01%, 0.05%, and 0.1% of A3, A4, and A5 and we used as blank the polymeric films without azo compound.

The antimicrobial effect of the PLA-based films was tested analyzing the inhibition of biofilm formation of *S. aureus* and *Candida albicans* on the active films, by staining with crystal violet. In this kind of test, the crystal violet is able to attach to the biofilm surface, making the film surface purple colored. When the biofilm formation is inhibited, because of the presence of active azo dye on the polymer surface, the purple color is no more detectable and the natural yellow color of the azo dye is then visible on the film, depending on azo dye concentration.

S. aureus strain A170 cells ($\sim 10^4 - 10^5$ CFU/mL) were suspended in Mueller-Hinton Broth (MHB) and aliquoted in a 24-well plate. Portions of the PLA films (1 cm^2) were added to each well and incubated for 24 h at 37°C. Films were moved in a new 24-well plate and washed three times with phosphate-buffered saline (PBS, pH 7.2) to remove nonadherent cells. The remaining biofilm was dried and stained with crystal violet solution 0.3% (w/v) for 15 min. After rinsing with water, to remove unbound crystal violet, and drying, the films were photographed to allow a qualitative comparison between tested films.

For control experiments 1 cm^2 of the polymeric films without azo compound was incubated in MHB medium with (K) and without *S. aureus* cells (K′).

The same protocol was used for the *C. albicans* biofilm inhibition. *C. albicans* cells were suspended in 200 μL RPMI Medium 1640 at 10^6 cells/mL, seeded in a flat-bottom 24-well plate with a portions of films (1 cm^2) and incubated 48 h at 37°C. Films were moved in a new 24-well plate and washed to remove nonadherent cells afterward stained with crystal violet solution 0.3% (w/v). After rinsing with water and drying, the films were photographed to allow a qualitative comparison between tested films.

For control experiments 1 cm^2 of the polymeric films without azo compound was incubated in RPMI plus *C. albicans* (K) or in RPMI without *C. albicans* (K′).

***In Vitro* Azo Compound Release Study.** We chose to analyze the release from films with the highest azo compound content (0.1% w/w) and we detected the concentration of released azo molecules by UV–Vis absorption spectra recorded as a function of time.

Dried PLA films loaded with 0.1% of azo compounds were cut into 1 cm^2 pieces. Each part was immersed into 3 mL of PBS buffer (pH 7.4) in a cuvette suitable for the UV-Vis instrument. The wavelengths used for the detection of molecules were: 358 nm for A3 and 357 nm for A4 and A5, according to their absorption maxima.[7]

After 8 h, the PLA films were removed from PBS buffer, and vacuum-dried for 24 h. Each film was then completely dissolved into 3 mL of chloroform/acetonitrile solution (2 : 5 v/v) by mixing for 2 min at room temperature. The remaining azo dye content in the film was determined by optical absorbance.

The results were expressed as a percentage of the azo released, according to the following equation:

$$\%\ \text{azo released} = \frac{\text{Absorbance of azo released at time } t}{\text{Absorbance of total azo incorporated}} \times 100$$

The percentage of released azo dye was calculated as the ratio of the absorbance value at λ_{max} of azo dye released during 8 h of analysis and the absorbance value at λ_{max} of total amount of azo dye incorporated in the PLA film.

RESULTS AND DISCUSSION

Thermal and Structural Characteristics of the Films

We performed thermogravimetric (TGA) and calorimetric (DSC) analyses to study the thermal behavior of the films and to evaluate the structural changes introduced by azo compounds on the polymers. Dried films were observed under polarized light, in order to confirm the amorphous nature of the material and to exclude the presence of air bubbles or crystals of azo compound in the matrix; this last aspect was also confirmed by X-ray analysis.

As representative examples, in Figure 1 we report the results of the X-ray analyses made on the blank PLA 4032D matrices and on PLA 4032D film (prepared by melt compounding method) containing the highest percentage of A4 azo compound. In particular, the diffraction patterns of film of PLA 4032D (a), film of PLA 4032D with 0.1% of A4 (b), and A4 crystals (c), registered at the same sample-to-camera distance of 9 cm, are qualitatively compared in Figure 1. For both polymer films the X-rays show only the amorphous halo, in contrast to a crystalline pattern visible for A4. This evidence confirms that there are no crystals of A4 in the matrix: this suggests that A4 is homogeneously dispersed in the polymer. Moreover, Figure 1 shows that there is no trace of crystallinity in PLA films, in spite of the tendency of PLA to crystallize under typical extrusion conditions; this was also confirmed by the microscope observations and from the complete transparency and homogeneity of all film samples.

Azo-compound A4 and PLA 4032D film were also analyzed by WAXD, to obtain complete X-ray diffraction peak patterns. For the PLA films\again, only the amorphous halo was registered by X-ray. The A4 crystalline reflection peaks (2θ) are reported in the Table I.

Thermal characterizations indicated that the presence of the highest percentage of azo dye in the matrix (0.1%) does not influence the thermal behavior of the material. In Table II we summarized the case of PLA film containing A4 as a representative example. Thermogravimetric curves of the blank and the azo containing film were completely overlapped, giving rise to the same degradation profile. Changes in glass transition temperature were very limited. In contrast, as expected, the thermal proprieties were altered from the presence of plasticizers: the presence of plasticizers decreases the T_g values of the pure PLA matrices.

Table I. X-ray Diffraction Peaks of A4

Peak number	2θ (deg)	Intensity (a.u.)
1	6.42	18,983
2	9.57	16,742
3	15.29	4183
4	15.79	10,528
5	18.03	4071
6	18.50	4110
7	20.94	3856
8	24.22	3020
9	25.80	3185
10	26.71	2894

Table II. Thermal Characteristics of the Films

Film	T_g [a](°C)	T_d [b](°C)	Preparation method
PLA 4060D/Tween 80 + 0.1% of A4	33.9	305	SC
PLA 4060D/Tween 80	33.9	305	
PLA 4060D/4032D/ Tween 80 + 0.1% of A4	40.0	309	SC
PLA 4060D/4032D/ Tween 80	40.9	309	
PLA 4032D + 0.1% of A4	59.6	318	MC
PLA 4032D	60.9	318	

[a] T_g = glass transition temperature obtained by DSC;
[b] T_d = 5% weight loss temperature obtained by TGA;
SC = solvent casting; MC = melt compounding.

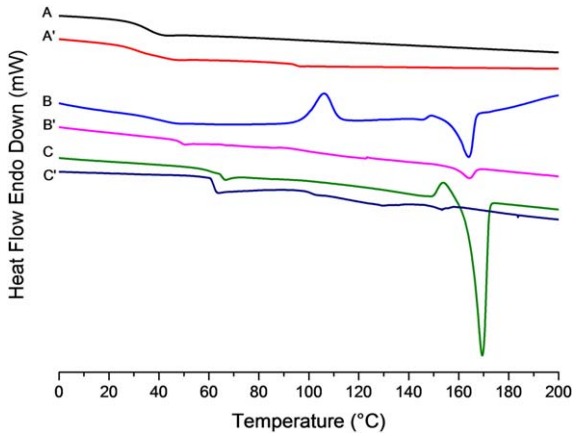
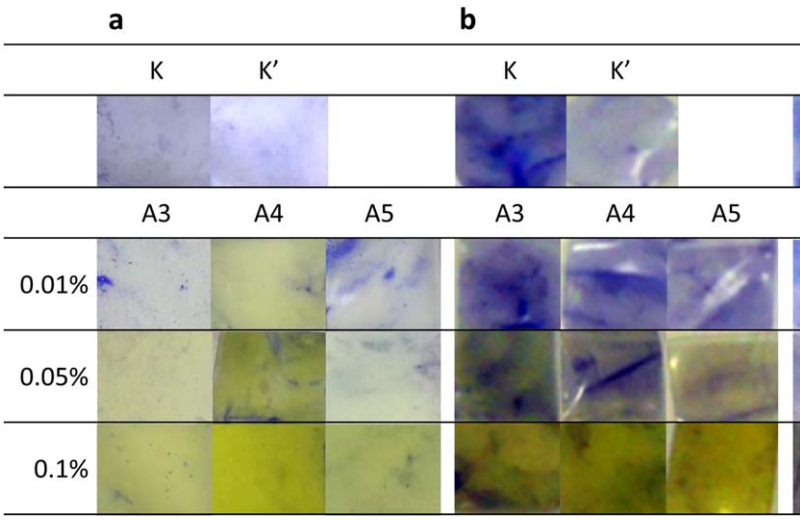


Figure 2. DSC thermograms of the representative PLA films with and without the addition of azo dyes, registered by DSC at 10°C/min, under nitrogen flow. Curves: A = PLA 4060D/Tween 80; A' = PLA 4060D/Tween 80 + 0.1% of A4; B = PLA 4060D/4032D/Tween 80; B' = PLA 4060D/4032D/Tween 80 + 0.1% of A4; C = PLA 4032D; C' = PLA 4032D + 0.1% of A4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Thermal behavior is summarized in Figure 2 for representative films of PLA with 0.1% of A4. As expected, for amorphous films based on PLA 4060D only the T_g is visible in the thermogram, and the presence of A4 at the maximum concentration (0.1%) does not influence the thermal behavior. For films containing PLA 4032D (curves B and C), besides the glass transition, a re-crystallization occurs by heating, followed by the melting of the crystalline fraction of the PLA. This crystallization is mostly hampered by the presence of 0.1% of azo dye (curves B′ and C′), indicating that the presence of small molecules has a plasticizing effect on the polymer.

Mechanical Properties

We performed dynamic mechanical (DMA) analyses to study the mechanical behavior of the films and to evaluate the changes introduced by azo compounds on the polymers. In Figure 3, we report the results of the DMA analyses made on the blank PLA 4032D matrices and on PLA 4032D and 4060D film. In particular, the storage and the loss moduli, shown in Figure 3, show a negligible influence of processing by addition of Tween 80 and azo compounds on the polymers.



Hydrophobic Properties

Contact angle measurements provide a better understanding of the interactions between solids and liquids. In this work, we measured contact angle and the wetting tension of PLA films in order to study the interaction of film surfaces with water and to evaluate how the insertion of azo dyes in PLA matrices can affect the biofilm formation ability.

The liquid barrier properties of the samples were assessed by static average Contact Angle (CA) measurements. In Table III the average water CA values and the wetting tension are reported for PLA 4060D/4032D/Tween 80 (blank) and PLA 4060D/4032D/Tween 80 + 0.1% of A5; PLA 4060D/Tween 80 (blank) and PLA 4060D/Tween 80 + 0.1% of A5, all obtained by solvent casting, and PLA 4032D, PLA 4032D + 0.1% of A5 obtained by melt compounding-compression molding method.

First, the obtained data suggest that the preparation method changed the surface proprieties of the PLA-based films. As expected the solvent casting method produces more porous surfaces, depending on the solvent evaporation through the polymeric matrix. Because of this different surface morphology, the contact angle measurements were not fully reproducible, showing an high deviation standard values for solvent-casted films.

Table III. Static Average Contact Angle (CA) for Water on Film Surface of Three PLA Films Before and After the Addition of A5 Azo Dye

Film	Static average CA on films side [°][a]	Wetting Tension [dy/cm][a]	Film preparation method[b]
PLA 4060D/Tween 80	49 ± 8	47.7 ± 7.5	SC
PLA 4060D/Tween 80 + 0.1% of A5	55 ± 10	41.9 ± 10.2	
PLA 4060D/4032D/Tween 80	48 ± 9	48.6 ± 8.1	SC
PLA 4060D/4032D/Tween 80 + 0.1% of A5	48 ± 6	48.8 ± 5.4	
PLA 4032D	65 ± 5	31.15 ± 5.7	MC
PLA 4032D + 0.1% of A5	76 ± 1	17.72 ± 1.3	

[a] Deviation standard values for five measurements.
[b] [b]SC, solvent casting; MC, melt compounding.

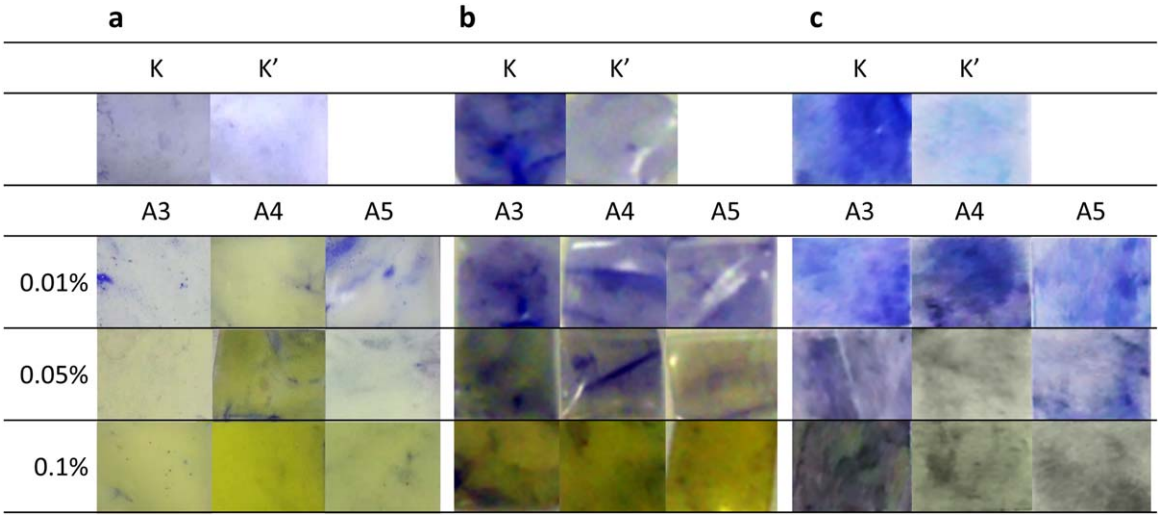


Figure 4. *S. aureus* biofilms formation on (a) solvent-casted PLA 4060D/4032D/Tween 80; (b) solvent-casted PLA 4060D/Tween 80; (c) mold-casted PLA 4032D film. K = positive control, K′ = negative control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In Table III, it may be noticed that the melt compounding/compression molding preparation method determined an increase of CA value associated to the minimum standard deviation value, suggesting that the surface of mold-casted films is more homogenous than solvent-casted films surface.

Moreover, the presence of azo-compound caused an increase of the average CA, matching with a decrease of wetting tension. This means that in this case the film surface is more hydrophobic, which can affect the ability of the material surface to interact with a biofilm. Our results suggest that the films with azo dye will be less prone to the attachment of bacterial or fungal biofilm.

Antimicrobial Test Results

We analyzed the inhibition of *S. aureus* and *C. albicans* biofilm formation on biodegradable films by staining with crystal violet. Figure 4 shows the formation of *S. aureus* biofilm on PLA film surface (K), and on PLA film surfaces with 0.1%, 0.5%, and 0.01% of azo dyes A3, A4, and A5. The blue-violet color is indicative of bacterial biofilm formed on the polymer film. At a concentration of 0.1% of azo compounds the biofilm formation was completely inhibited; this is evident by the complete blue discoloration of these films. In particular, the best antibacterial agent was found to be A4 into PLA 4060D/4032D/Tween 80 matrix, Figure 4(a), which showed a discoloration comparable to the K′ control (PLA film incubated with MHB medium without *S. aureus*), indicating a complete inhibition of *S. aureus* biofilm formation even at 0.01%. Into PLA 4060D/Tween 80 matrix, Figure 4(b), all azo compounds showed partial inhibition of *S. aureus* biofilm formation at 0.05%. In the PLA 4032D mold-casted film, it was very hard to discern the best antibacterial agent, but A4 showed partial inhibition of *S. aureus* biofilm formation at 0.05%.

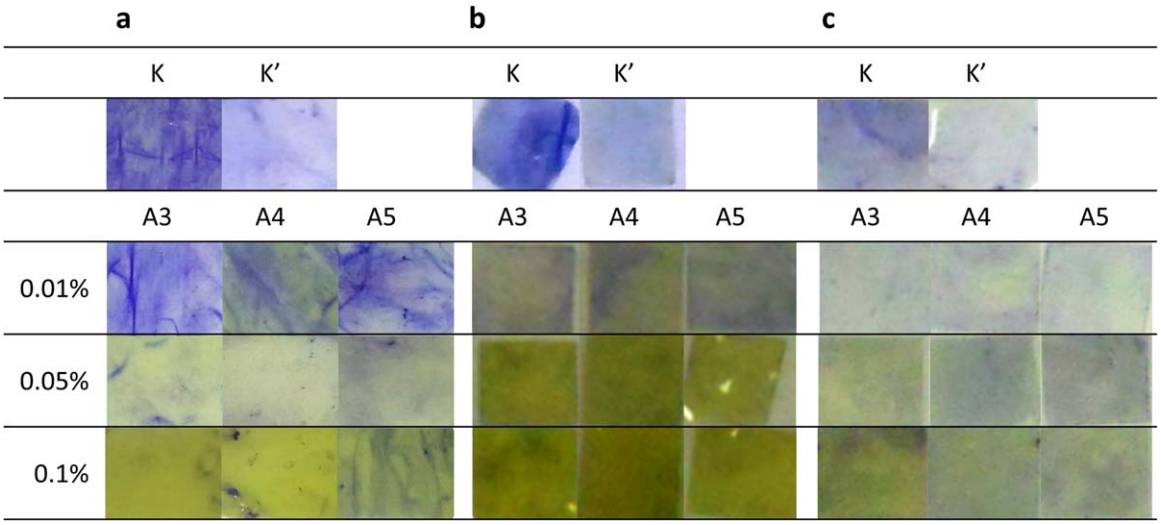


Figure 5. *C. albicans* biofilms formation on (a) solvent-casted PLA 4060D/4032D/Tween 80; (b) solvent-casted PLA 4060D/Tween 80; (c) mold-casted PLA 4032D film. K = positive control, K' = negative control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

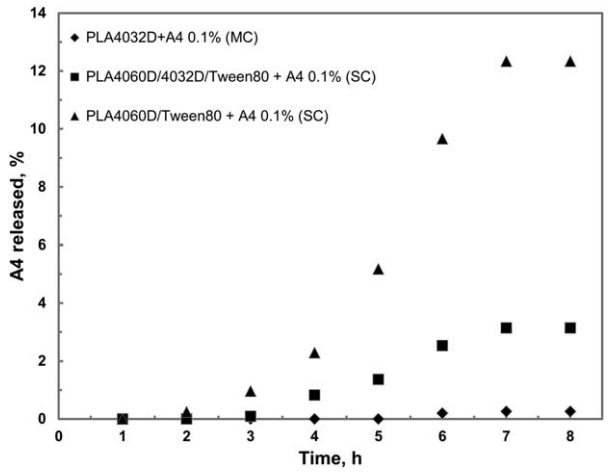


Figure 6. Release of azo dye A4 from three different PLA films obtained by solvent casting (SC) or melt.

Figure 5 shows the results of inhibition of *C. albicans* biofilm formation on the surface of PLA-based films, analyzed by the same qualitative colorimetric method. For each azo compound, a good activity was observed starting from 0.05% of azo compounds.

In particular, in PLA 4060D and PLA 4032D matrices all azo dyes A3, A4, and A5 showed a partial inhibition of *C. albicans* biofilm formation at activity even at 0.01%, as confirmed by the partial blue discoloration [Figures 5(b,c)].

Remarkably, the azo dye concentration of polymer films that is sufficient to give the polymer films a good antimicrobial activity, are considerably lower than typical concentrations reported in literature.[1] This is very promising for the industrial use of these materials.

Azo Dye Release

The study of release of azo dyes from all the polymer films is summarized in Figure 6 and in Figures S1 and S2 of Supporting Information. Release of azo molecules from the polymer films reached a plateau within 8 h. After 8 h, the polymer films appeared undamaged and with the same initial mechanical properties.

As example, the percentage of A4 released from PLA-based films was plotted as a function of time in Figure 6.

The first general consideration that arises from these results is that the release of azo compounds from PLA-based films produced by solvent casting (SC) is faster than release from films prepared by melt compounding process (MC).

Also, a different behavior of the two films prepared by solvent casting is observed: the one formed by PLA 4060D/Tween 80 releases more than the films of PLA 4060D/4032D/Tween 80.

The nature of the PLA could be responsible for this behavior. In fact, PLA 4060D, which is completely amorphous, is the one that releases most (triangle points in Figure 6). In the films processed by MC method, the presence of PLA 4032D, which shows a tendence to semicrystalline structure, leads to a slower release of A4. This release behavior is confirmed also by the azo compounds A3 and A5 (see Figures S1 and S2 of Supporting Information).

These results demonstrate that it is possible to tune the kinetic of release changing PLA structure and film preparation method.

CONCLUSIONS

In this work we presented innovative biodegradable antimicrobial films, consisting of azo dyes blended in PLA matrices, realized with two different processing techniques. Azo containing PLA films are capable to inhibit *S. aureus* and *C. albicans* biofilm formation. This effect is enhanced by a decrease in wettability of the surface, because of the presence of azo dyes in the film. In particular, *C. albicans* biofilm formation is inhibited on the surface of all films at a concentration of 0.05% of azo compounds A3, A4, and A5, whereas A4, the most active antibacterial agent, showed a complete inhibition of *S. aureus* biofilm formation at 0.01% in PLA film prepared by solvent casting. These concentrations of antimicrobial agents are low enough to permit the preparation of transparent and homogeneous polymer films. The rate of release of the active molecules form the different PLA films is depending on the structure and on the preparation process of PLA. High release rate is easily obtained increasing the amount of amorphous PLA 4060D in the film. The antimicrobial activity against two demonstrative organisms renders the resulting materials promising for biomedical and antimicrobial active packaging applications.

REFERENCES

1. Muñoz-Bonilla, A.; Fernández-García, M. *Prog. Polym. Sci.* **2012**, *37*, 281.
2. Kenawy, E.-R.; Worley, S.; Broughton, R. *Biomacromolecules* **2007**, *8*, 1359.
3. Siedenbiedel, F.; Tiller, J. C. *Polymers* **2012**, *4*, 46.
4. Woo, G.; Mittelman, M.; Santerre, J. *Biomaterials* **2000**, *21*, 1235.
5. Knetsch, M. L.; Koole, L. H. *Polymers* **2011**, *3*, 340.
6. Kenawy, E. R.; Abdel-Hay, F. I.; El-Shanshoury, A. E. R. R.; El-Newehy, M. H. *J. Polym. Sci. Part A Polym. Chem.* **2002**, *40*, 2384.
7. Piotto, S.; Concilio, S.; Sessa, L.; Porta, A.; Calabrese, E. C.; Zanfardino, A.; Varcamonti, M.; Iannelli, P. *Eur. J. Med. Chem.* **2013**, *68*, 178.
8. Concilio, S.; Piotto, S.; Sessa, L.; Iannelli, P.; Porta, A.; Calabrese, E. C.; Galdi, M. R.; Incarnato, L. *AIP Conf. Proc.* **2012**, *1459*, 256.
9. Piotto, S.; Concilio, S.; Sessa, L.; Iannelli, P.; Porta, A.; Calabrese, E. C.; Galdi, M. R.; Incarnato, L. *Polym. Compos.* **2013**, *34*, 1489.
10. Auras, R. A.; Lim, L.-T.; Selke, S. E.; Tsuji, H. Poly (Lactic Acid): Synthesis, Structures, Properties, Processing, and Applications; John Wiley & Sons: New York, **2011**.
11. Garlotta, D. *J. Polym. Environ.* **2001**, *9*, 63.
12. Sellenet, P. H.; Allison, B.; Applegate, B. M.; Youngblood, J. P. *Biomacromolecules* **2007**, *8*, 19.

13. Kolstad, J. J. *J. Appl. Polym. Sci.* **1996**, *62*, 1079.
14. Ignjatovic, N.; Uskokovic, D. *Appl. Surf. Sci.* **2004**, *238*, 314.
15. Suuronen, R.; Pohjonen, T.; Hietanen, J.; Lindqvist, C. *J. Oral Maxillofac. Surg.* **1998**, *56*, 604.
16. Bergsma, J.; Rozema, F.; Bos, R.; Boering, G.; De Bruijn, W.; Pennings, A. *Biomaterials* **1995**, *16*, 267.
17. Singh, S.; Ray, S. S. *J. Nanosci. Nanotechnol.* **2007**, *7*, 2596.
18. Piotto, S.; Concilio, S.; Mavelli, F.; Iannelli, P. *Macromol Symp.* **2009**, *286*, 25.
19. Saeidlou, S.; Huneault, M. A.; Li, H.; Park, C. B. *Prog. Polym. Sci.* **2012**, *37*, 1657.
20. Wang, S.; Zhang, Y.; Wang, H.; Yin, G.; Dong, Z. *Biomacromolecules* **2009**, *10*, 2240.
21. Gahleitner, M.; Grein, C.; Kheirandish, S.; Wolfschwenger, J. *Int. Polym. Process.* **2011**, *26*, 2.
22. Xu, H.; Xie, L.; Chen, J.-B.; Jiang, X.; Hsiao, B. S.; Zhong, G.-J.; Fu, Q.; Li, Z.-M. *Materials Horizons (Royal Society of Chemistry)* **2014**.
23. Jin, T.; Liu, L.; Zhang, H.; Hicks, K. *Int. J. Food Sci. Technol.* **2009**, *44*, 322.
24. Jin, T.; Zhang, H. *J. Food Sci.* **2008**, *73*, M127.
25. Özge Erdohan, Z.; Çam, B.; Turhan, K. N. *J. Food Eng.* **2013**, *119*, 308.
26. Mensitieri, G.; Di Maio, E.; Buonocore, G. G.; Nedi, I.; Oliviero, M.; Sansone, L.; Iannace, S. *Trends Food Sci. Technol.* **2011**, *22*, 72.
27. Gorrasi, G.; Pantani, R. *Polym. Degrad. Stab.* **2013**, *98*, 1006.
28. Speranza, V.; De Meo, A.; Pantani, R. *Polym. Degrad. Stab.* **2014**, *100*, 37.
29. Vieira, M. G. A.; da Silva, M. A.; dos Santos, L. O.; Beppu, M. M. *Eur. Polym. J.* **2011**, *47*, 254.