New PLA/ZnO:Cu/Ag bionanocomposites for food packaging

C. Vasile¹, M. Răpă^{2*}, M. Ștefan³, M. Stan³, S. Macavei³, R. N. Darie-Niță¹, L. Barbu-Tudoran³, D. C. Vodnar⁴, E. E. Popa⁵, R. Ștefan⁴, G. Borodi³, M. Brebu¹

¹ ‘Petru Poni’ Institute of Macromolecular Chemistry, Physical Chemistry of Polymers Department, 41A Gr. Ghica Voda Alley, 700487 Iasi, Romania

²Research Institute of Auxiliary and Organic Products S.A. (ICPAO), 8 Carpati St., 551022 Medias, Sibiu, Romania

³National Institute for Research and Development of Isotopic and Molecular Technologies INCDTIM, 67–103 Donath St., 400293 Cluj-Napoca, Romania

⁴University of Agronomic Sciences and Veterinary Medicine Cluj-Napoca, Department of Food Science and Technology, 3–5 Manastur Street, 400372 Cluj-Napoca, Romania

⁵University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Biotechnology, 59 Marasti Blv., district 1, 011464 Bucharest, Romania

Received 21 November 2016; accepted in revised form 26 January 2017

Abstract. Plasticized poly(lactic acid) (PLA) samples with embedded Cu-doped ZnO powder functionalized with Ag nanoparticles composites were prepared by melt blending processing technique. The effect of ZnO:Cu/Ag nanoparticles on the properties of plasticized PLA was investigated in terms of structural (by Attenuated total reflectance – Fourier Transform Infrared – ATR-FT-IR, X-Ray Diffraction analysis – XRD) and morphological (using Scanning Electron Microscopy – SEM and Transmission Electron Microscopy – TEM examination) modifications, thermal (Differential Scanning Calorimetry – DSC), mechanical (transmittance, tensile strength), barrier antimicrobial properties and also in respect to migration of Cu, Zn and Ag nanoparticles into food simulants. It was found that the increase of nanoparticle content (from 0.5 to 1.5 wt%) of PLA formulations leads to the increase of the degree of crystallinity of PLA. The overall migration of all samples into three food simulants was below $10 \text{ mg} \cdot \text{dm}^{-2}$, accepted value according to EU Regulation No 10/2011 for plastic materials and articles intended to come into contact with food. Based on the obtained results it can conclude that the optimum composition is PLA/ZnO:Cu/Ag 0.5, this bionanocomposite offering suitable mechanical and thermal properties, good barrier properties to ultraviolet light, water vapour, oxygen and carbon dioxide, antibacterial activity and low migration amount of nanoparticles into food simulants.

Keywords: nanocomposites, antibacterial agent, food packaging, material testing

1. Introduction

Nowadays, almost all aspects of daily life involve polymeric materials. With increasing environmental awareness around the world, the research and development of biodegradable polymers attracted a great attention [1–3].

Poly(lactic acid) PLA is one of the most popular commercial biodegradable, thermoplastic material being

derived from renewable resources. It was studied in combination with other renewable resources, such as cellulose, chitosan [4, 5] in particular for food packaging. The general properties required for biodegradable active food packaging materials envisage: antimicrobial function, mechanical, optical and thermal properties, gas barrier and biodegradability [6–8]. The main drawbacks of PLA to be used in food

*Corresponding author, e-mail: rapa_m2002@yahoo.com
© BME-PT

packaging are its low gas barrier properties, low processability and its quite slow crystallization rate. Different strategies have been used to improve its barrier properties including barrier surface film coating, multilayer coextrusion, plasma deposition, incorporation of nanoclays, or increasing of crystallization degree [9, 10]. Regarding the improvement of the PLA processability, many studies have been concerned on the use of bioplasticizers, such as lactic acid oligomers [11], byproducts rich in monoterpenes [12], etc. In the case of food packaging, special attention should be addressed to the volatiles and migration of the plasticizers and other compounds into foodstuff [13].

Organic compounds such as chitosan and its derivatives, essential oils, organic acids, enzymes like lysozyme and bacteriocins have been widely studied for their antimicrobial properties and tested for their potential application in polymeric matrices such as antimicrobial packaging. However, such organic compounds present high sensitivity to intense processing conditions (as high temperatures and pressures) necessary in most industrial technologies. The development of microorganisms' resistance also leads to find new solutions for food packaging [14].

Some inorganic compounds at a nanosize scale present strong antibacterial activities at low concentrations due to their high surface area to volume ratio and unique chemical and physical properties. Most antibacterial inorganic materials are metallic nanoparticles and metal oxide nanoparticles such as silver, copper, titanium oxide and zinc oxide, magnesium and calcium oxide, etc. Through the incorporation of metal nanoparticles (NPs) within the polymer matrices versatile materials with interesting properties useful for food packaging applications can be obtained [15]. They are also more stable under extreme conditions such as high temperature and pressures and some of them are considered nontoxic and even contain mineral elements essential to human health.

ZnO is generally recognized as safe (GRAS) material by the Food and Drug Administration (FDA) and is used as food additive. ZnO at nanoscale level shows antimicrobial properties and potential applications in food preservation. ZnO nanoparticles have been incorporated in polymeric matrices in order to provide antimicrobial activity and to improve packaging properties [16]. It was incorporated in polycarbonate and the hydrophobic nanocomposites obtained showed antibacterial, UV-blocking properties [17]. Glass,

low density polyethylene (LDPE), polypropylene (PP), polyurethane (PU), paper and chitosan, polyethylene [18] and polypropylene [19] were also coated with ZnO nanoparticles to obtain antimicrobial food packagings.

Silver is well-known for its wide-range antimicrobial activity against both Gram-positive and Gram-negative bacteria (including antibiotic-resistant strains), fungi and certain viruses. Silver nanoparticles (nanosilver) and copper nanoparticles (nanocopper) exhibit antimicrobial activity and have been incorporated into polymers as polyolefin nanocomposites [20], hydroxypropyl methylcellulose [21] to create antimicrobial packaging materials.

The copper composites are effective against pathogens and bacteria. It can work against viruses and fungi, and other bacteria that cause food poisoning, such as *Listeria* and *Salmonella*, and Methicillin resistant *Staphylococcus aureus* (MRSA), a bacteria that is particularly difficult to treat with antibiotics. Copper nanoparticles embedded in polylactic acid, combining the antibacterial properties of copper nanoparticles with the biodegradability of the polymer matrix [22].

PEG-plasticized PLA-based composite films containing ZnO (<50 and <100 nm), or Ag–Cu alloy were prepared by a solvent casting method [23, 24]. The obtained films show the increased glass transition temperature, thermal stability, crystallinity, much higher tensile strength than that of PLA/PEG blend. The mechanical property drastically reduced with a loading of NPs, which is associated with degradation of PLA. SEM micrographs examination shown that both Ag–Cu alloy or ZnO NPs were dispersed homogeneously in the PLA film matrix.

In case of the materials designed to food packaging application, there are some concerns regarding the migration of ZnO, Ag and Cu nanoparticles to food. Migration of silver ranged from 0.003 to 0.005 mg/dm², while migration of copper ranged from 0.024 to 0.049 mg/dm² [20].

Taking into account the antimicrobial activity of ZnO, CuO and Ag nanoparticles it can be supposed that their combination will widen the antimicrobial spectrum and also the improvement of some of properties necessary to food packaging will be obtained by using a commonly applied technology as melt processing. The aim of this work was to investigate the influence of Cu-doped ZnO nanoparticles functionalized with Ag nanoparticles on the properties of

plasticized with tributyl *o*-acetyl citrate/Lapol 108 masterbatch PLA polymer intended to obtain new food packaging materials. To our knowledge, the incorporation of nanoparticles (NPs) like ZnO:Cu/Ag alloy as nano-scale fillers into PLA matrix by melt mixing procedure had never been reported before. It was expected that the three-metals (ZnO with bimetallic NPs (Cu and Ag)) containing nanoparticles incorporation into PLA matrix plasticized by melt mixing method confers to the final PLA-based materials good barrier and antibacterial properties as well as low migration of nanoparticles into packaged food.

2. Experimental part

2.1. Materials

Poly(lactic acid (PLA) Ingeo™ biopolymer 2003D purchased from NatureWorks LLC (Minnetonka, USA), characterized by a residual monomer content of 0.21%, relative viscosity of 3.92 and a *D*-lactide content of 4.5% was used in this study. It is a thermoplastic resin derived from annually renewable resources and is accepted by the Food & Drug Administration (FDA) to be used in food packaging.

As bioplasticizers were used: tributyl *o*-acetyl citrate (ATBC) – Proviplast 2624 supplied by Proviron, Belgium (Hemiksem, Belgium) (molecular weight of 406 g/mol, density at 25 °C of 1.055 kg/l and assay (GC) 99.35%) and Lapol 108 masterbatch (MB) (as 30% Lapol 108 in PLA) purchased from Lapol, LLC (Santa Barbara, USA).

Zinc nitrate hexahydrate – $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (98% Sigma Aldrich, St. Louis, Missouri; USA), copper nitrate trihydrate – $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (99.5%, Merck, Darmstadt, Germany), oxalic acid dehydrate – $\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ (Anala™ Normapur, VWR, Prolabo), silver nitrate – AgNO_3 (Sigma Aldrich, St. Louis, Missouri, USA), absolute ethanol (S.C. Nordica S.R.L., Romania) and aqueous solution of *Aloe vera* leaf extract obtained in our own laboratory were used to obtain ZnO:Cu/Ag nanoparticles.

2.2. Synthesis of Cu-doped ZnO nanoparticles

The copper-doped zinc oxide powders, $\text{Zn}_{1-x}\text{Cu}_x\text{O}$ ($x = 0.01$), were synthesized by co-precipitation using a modified version of the experimental procedure reported by Brihi *et al.* [25]. For this purpose, an oxalate precursor was obtained by co-precipitation of a $0.4 \text{ mol} \cdot \text{L}^{-1}$ aqueous solution of zinc and copper nitrates [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$], respectively, with the same volume of a $0.4 \text{ mol} \cdot \text{L}^{-1}$

solution of oxalic acid ($\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$). The reaction mixture was stirred for 1 h at room temperature. The light blue precipitate was thoroughly washed with ultrapure water and dried at room temperature. After drying, it was grounded to give fine powder and annealed at 450 °C (heating rate of 5 °C/min) for 2 h in air.

2.3. Functionalization of Cu-doped ZnO powder with Ag nanoparticles

The aloe leaf extract mediated green synthesis of silver nanoparticles was applied. The leaf extract of *Aloe vera* was prepared according to the following procedure: 20 g of fresh leaves were finely sliced and extracted in 100 mL ultrapure water at 60–80 °C with magnetic stirring at 900 rpm for 20 min. The extract was cooled down to room temperature and filtered, then its volume was adjusted to 100 mL by washing the vegetal residue with ultrapure water, and was further used for functionalization of the nanopowders.

Functionalization of Cu-doped ZnO powder with AgNPs was performed following the procedure reported by Vivekanandhan *et al.* [26]. For this purpose, the Cu-doped ZnO powder (2 g) was dispersed in 500 mL of $2 \cdot 10^{-3} \text{ M}$ AgNO_3 solution. The obtained dispersion was sonicated for 2 h, and then 25 mL of *Aloe vera* leaf extract was added under continuous stirring with a rate of 500 rpm at room temperature. A change in the reaction mixture colour into brown was observed after 30 min, indicating the formation of silver nanoparticles. The stirring was continued for 24 h in order to complete the reduction reaction. The Cu-doped ZnO functionalized with Ag nanoparticles (ZnO:Cu/Ag NPs) obtained were collected through centrifugation (7000 rpm, 15 min, 21 °C). Finally, they were washed several times with absolute ethanol to eliminate the bioresidues and dried at room temperature prior to characterization.

The ZnO:Cu/Ag nanoparticles morphology was examined by SEM/TEM analysis (see below Figure 3j) and was found a mean size of ZnO:Cu/Ag NPs ranging from ~15–24 nm (Figure 1).

In all characterizations, the functionalized Cu-doped ZnO powder with Ag nanoparticles was coded as ZnO:Cu/Ag.

2.4. Preparation of PLA bionanocomposites

The ATBC/MB plasticized PLA/ZnO:Cu/Ag bionanocomposites containing 0, 0.5, 1 and 1.5 wt% Cu-doped ZnO powder functionalized with Ag nanoparticles were prepared by melt blending on a Brabender

Figure 1. The particle size distribution of ZnO:Cu/Ag nanoparticles evaluated by TEM analysis. N/N_0 – number of particles with certain dimension/total number of particles

Plastograph (Duisburg, Germany) at a temperature of 170 °C and 60 rpm. The samples codes as well as their composition are given in Table 1. The ratios between PLA and plasticizers and MB and ATBC were kept constant at 80:20 and 3:1 (wt%), respectively. Square sheets with (150×150×1) mm dimensions and films with thickness below 200 µm were prepared from the melted blends by pressing at a temperature of 175 °C, preheating time of 4 min, pressing time of 2 min, under a pressure of 150 Pa and 125 Pa respectively, for films and sheets (thickness of 1 mm), followed by a sudden cooling of the mold under pressure. The specimens for characterization were taken out from these sheets and films. Neat

Table 1. Studied samples and composition of PLA bionanocomposites

No Crt	Sample Code	PLA [wt%]	MB+ATBC [wt%]	ZnO:Cu/Ag [wt%]
1	PLA	100.0		
2	PLA/ZnO:Cu/Ag 0	80.0	20.0	
3	PLA/ZnO:Cu/Ag 0.5	79.6	19.9	0.5
4	PLA/ZnO:Cu/Ag 1	79.2	19.8	1.0
5	PLA/ZnO:Cu/Ag 1.5	78.8	19.7	1.5
6	ZnO:Cu/Ag			100.0

Figure 2. The aspects of PLA/ZnO-Cu/Ag bionanocomposites films with different content of nanoparticles (0; 0.5; 1 and 1.5%)

PLA was used as reference for FT-IR, DSC, barrier and mechanical properties.

The aspects of prepared films of some bionanocomposites in comparison with plasticized PLA are shown in Figure 2.

By ZnO:Cu/Ag nanoparticles incorporation into plasticized PLA, the brown color is more intense due to the increased AgNPs content. However, all films are transparent, the lines from background being visible (Figure 2).

2.5. Investigation methods

2.5.1. Transmission/scanning electron microscopy (TEM/SEM) and EDX analysis

The morphology of samples was investigated by transmission/scanning electron microscopy (TEM/SEM) using a Hitachi SU8230 High Resolution Scanning electron Microscope (Tokyo, Japan) equipped with a cold field emission gun. The morphology of the ZnO:Cu/Ag nanoparticles was determined by transmission electron microscopy (TEM). Here, the ZnO:Cu/Ag nanoparticles were dispersed in ethanol, with a BANDELIN SONOREX homogenizer GM 3100 (Berlin, Germany). Fourier analysis of images was made with Digital Micrograph for GMS by Gatan software. For scanning electron microscopy (SEM) analysis the fractured surfaces of composite films was deposited on aluminium stubs and sputter-coated with 10 nm gold on a Q150T ES Quorum (Oxford, UK).

Elemental composition of the samples was determined by energy dispersive X-ray analysis (EDX), with an EDX-system (X-Max N80TLE Silicon Drift Detector (SDD)) from Oxford Instruments (Oxford, UK). Due to the high voltage of electron beam the samples were melted, complicating the surface analysis.

2.5.2. Attenuated total reflectance Fourier Transform (ATR-FTIR) spectroscopy

PLA/ZnO:Cu/Ag bionanocomposites films were analyzed in Attenuated Total Reflectance mode Fourier Transform infrared spectroscopy (ATR-FTIR) by using a FTLA 2000–104 spectrophotometer (ABB, Bomem Inc, Québec, Canada) equipped with a ZnSe crystal with an incidence angle of 45°. All spectra represent the average of 20 scans recorded at 4 cm⁻¹ resolution in a 2000 to 750 cm⁻¹ range, using air as background. The average FTIR spectra of the three measurements of the PLA/ZnO:Cu/Ag biocomposite films were normalised to a reference band at the

wave number of 2945 cm^{-1} of plasticized PLA. The processing of spectra was achieved by using the SpecView program.

2.5.3. Differential scanning calorimetry measurements (DSC)

Thermal analysis of PLA/ZnO:Cu/Ag bionanocomposites was performed using a DSC 823^e from Mettler Toledo (Greifensee, Switzerland) calibrated with indium. The weighed samples between 6 and 9 mg were packed in aluminum pans and placed in the DSC cell. The samples were first heated from $30\text{ }^{\circ}\text{C}$ to $190\text{ }^{\circ}\text{C}$, kept 2 min for remove of thermal history, cooled to $30\text{ }^{\circ}\text{C}$ and reheated to $190\text{ }^{\circ}\text{C}$. A heating rate of $5\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ was used for all processes. From DSC curves, second run, the melting temperature [T_m], enthalpy of melting [ΔH_m], cold crystallization temperature and [T_{cc}] and its enthalpy and degree of crystallinity [χ_c] were estimated from all samples.

The degree of crystallinity [χ_c] of the PLA samples was evaluated by dividing the melting enthalpy of the sample, ΔH_m [J/g] to the enthalpy value for a theoretically 100% crystalline PLA ($\Delta H_m^0 = 93\text{ J/g}$ [27]). The weight fraction of PLA from each sample was used for χ_c calculation.

2.5.4. X-ray diffraction analysis (XRD)

Structural characterization and crystallinity degree of polymer samples embedded with nanoparticles were investigated by using a D8 Advance X-ray powder diffractometer (Bruker, Germany), equipped with Ge(111) in the incident beam, a fast LynxEye detector and a Cu-K α 1 radiation source ($\lambda = 1.54060\text{ \AA}$), operating at 40 kV and 40 mA, as the applied voltage and intensity, respectively, over the incidence angle (2θ) in the range of $3\text{--}85^{\circ}$, at ambient temperature.

2.5.5. Transmittance by UV/VIS

Each film specimen was cut into a rectangular shape and placed directly in a UV/VIS HELIOS ALPHA Spectrophotometer (Thermo Spectronic, Cambridge, UK) at the wavelength from 325 to 800 nm. Measurements were performed using air as reference. The results have been expressed as percentage transmittance. The measurements were done in triplicate and the average of three spectra was calculated.

2.5.6. Tensile properties

The tensile strength at break, elongation at break and Young modulus were carried out on an Instron 3345

(USA) test machine with a load cell of 1 kN according to the ISO 527. At least five rectangular ($40\times 4\times 1$) mm test specimens were tested for each composition, at a crosshead speed of $10\text{ mm}\cdot\text{min}^{-1}$ and the average value was reported.

2.5.7. Barrier properties

Water vapor permeability

Water vapor transmittance rate (WVTR) of the samples was determined with PBI-Dansensor L 80–5000 (Ringsted, Denmark). The permeability of the samples was performed at $23\text{ }^{\circ}\text{C}$. Three determinations were made for each sample and the average value was reported.

Gas permeability test

The gas transmittance rate (of CO_2 and O_2) was conducted with a Lyssy L100–5000 (Systech Instruments Ltd, Illinois, UK) manometric gas permeability tester ($\sim 4\text{ h}$ to equilibrium for CO_2 and $\sim 7\text{ h}$ to equilibrium for O_2). Specimens with ($108\times 108\times 0.2$) mm dimensions were used. Four determinations were made for each sample and the average value was reported.

2.5.8. Migration of metals from composites formulations into simulant media

Metals release from composites formulations was performed by immersing into different simulant media a fractured surface with ($40\times 40\times 1$) mm dimensions from all PLA-based biocomposites into 100 ml simulant at $70\text{ }^{\circ}\text{C}$, for 2 h. Three types of simulant media were tested as follows: distilled water (simulant A), ethanol 10% (v/v) in aqueous solution (simulant B) and acetic acid 3% (w/v) in aqueous solution (simulant C). The concentrations of released Zn^{2+} , Cu^{2+} and Ag^{1+} in these simulants were determined by using an inductively coupled plasma atomic emission spectrometer (ICP-AES) (Perkin Elmer 5300 DV, Norwalk, CT, USA). Here, standard solutions with a concentration of $1000\text{ mg}\cdot\text{L}^{-1}$ of Zn, Cu and Ag ions were used for calibration.

2.5.9. Antibacterial activity

The bacterial adherence was tested to simulate the affinity of microorganisms to the material surface of 4 cm^2 . A protocol adapted from previous work [28] was applied for *Staphylococcus aureus* ATCC 49444 and *Pseudomonas aeruginosa* ATCC 27853 microorganisms. The bacteria were precultured from a frozen glycerol preparation and inoculated in 45 mL brain

heart infusion medium (BHI; Bacto™ Brain Heart Infusion, Becton, Dickinson & Company, Le Pont de Claix, France) for 16 h at 37 °C. After harvesting the bacteria by centrifugation (4000 rpm, + 4 °C, 10 min, Centrifuge 5810R, Eppendorf, Hamburg, Germany), they were washed once with phosphate-buffered saline (PBS) (diluted from Dulbecco's Phosphate-Buffered Saline (D-PBS) 10×, Invitrogen, Carlsbad, CA, USA). Then the cells were resuspended in PBS at a concentration of ~ 0.35 at $\lambda = 550$ nm, which corresponded to $\sim 1 \cdot 10^7$ colony-forming units (CFU) (measured with a Spectrophotometer BioSpec-mini DNA/RNA/protein analyzer, Shimadzu Biotech, Kyoto, Japan). The suspension was gently sonicated and vortexed to homogenize the solution. Then, the specimens were placed in test tubes (BD Falcon, 14 mL, Ref 352001, USA) with 5 mL of bacterial suspension. After rolling (Coulter mixer, Coulter electronics limited, Luton Bedfordshire, UK) at room temperature for 30 min, the specimens were washed three times in abundant physiological saline and gently dried without touching the surface. Thereafter, the bacterial samples from the specimen surfaces were collected for the viability analysis (three replicates). The bacteria attached to the surface of the specimens were collected with micro brushes into microtubes (2 mL, Sarstedt, Numbrecht, Germany) containing 900 μ L of Tryptic Soy Broth (TSB, Bacto™ Tryptic Soy Broth, Becton, Dickinson & Company, Wilson, NC, USA) with 10% glycerol. Thereafter, the bacteria were sonicated (~ 5 s with a tipsonicator type UP 50 H Ultrasonic Processor, Hielscher, Teltow, Germany cycle 1, amplitude 100%), serially diluted in physiological saline solution (10 μ L of 1:10, 1:100, and 1:1000) and cultured on blood agar plates. CFU measurements were done after 16 h of culturing at 37 °C.

3. Results and discussions

3.1. SEM analysis

The morphology of the films samples containing PLA, plasticizers and ZnO:Cu/Ag nanoparticles and ZnO:Cu/Ag nanoparticles was investigated using scanning electron microscopy – Figure 3. The plasticized PLA (Figure 3a) shows a smooth surface with very small pores of almost spherical shape, evidenced at high magnification (Figure 3b). The SEM images of biocomposite containing 0.5 wt% ZnO:Cu/Ag nanoparticles reveal the presence of spherical aggregates of about 50–100 nm, relatively uniform distributed on surface of polymer film (Figure 3c).

It can be noticed that aggregations tend to locate in pores of PLA matrix having a homogeneous dispersion in PLA matrix (Figure 3d). With the increase of the nanoparticles content (for 1 and 1.5 wt%) the tendency to aggregation is much pronounced and the distribution of ZnO:Cu/Ag nanoparticles is not more homogeneous (Figures 3e–3h).

The change in morphology is associated with the effect the ZnO:Cu/Ag nanoparticles. It is obvious that the incorporation of ZnO:Cu/Ag nanoparticles in PLA matrix promotes the reorganization of the matrix, in concordance with the DSC/XRD results reported in Table 3 and Table 4. The spatial homogeneous dispersion of elements and of the ZnO:Cu-Ag nanoparticles in PLA matrix and the quantitative composition of the film were confirmed by EDX analysis (Figures 4 and 5) and Table 2.

EDX spectra and mapping images allow highlighting the presence of Zn, O, Cu, Ag along with C from polymer matrix.

As concerns the surface composition of the PLA/ZnO:Cu/Ag 1.5 film it is seems that low amount of AgNPs is located on surface, while Zn and Cu are in a higher concentration.

3.2. ATR-FTIR spectroscopy characterization

Normalized ATR-FTIR spectra of the neat PLA, plasticized PLA and the PLA/ZnO:Cu/Ag biocomposite films are presented in Figure 6.

The bands in the ATR-FTIR spectra of PLA biocomposites assigned according to the literature data [29] are placed at following wave numbers: 1454 cm^{-1} (C–H stretching of $-\text{CH}_3$; asymmetric deformation), 1383 and 1360 cm^{-1} (C–H stretching of $-\text{CH}_3$; symmetric deformation), 1267, 1180, 1128, 1082 and 1041 cm^{-1} ($-\text{C}-\text{O}-$ stretching vibration in $-\text{O}-\text{C}=\text{O}$) and 868 cm^{-1} (C–O–C stretching). The band centered at 1749 cm^{-1} is assigned to the C=O stretching of the ester group of PLA. The weak bands around 2944 and 2995 cm^{-1} (not shown) are assigned to the C–H stretching of CH_3 .

Table 2. Elemental composition of the PLA/ZnO:Cu/Ag 1.5 film

Element	[wt%]
C	53.76
O	45.34
Cu	0.15
Zn	0.74
Ag	0.01
Total	100.00

Figure 3. SEM micrographs of the fractured surface of the PLA-plasticizer and PLA nanocomposites: a–b) Plasticized PLA; c–d) PLA/ZnO:Cu/Ag 0.5; e–f) PLA/ ZnO:Cu/Ag 1; g–h) PLA/ZnO:Cu/Ag 1.5; i–j) TEM micrographs of the ZnO:Cu/Ag NPs

A significant decrease in the intensity of bands was observed for PLA-bionanocomposites films containing 1 and 1.5% ZnO:Cu/Ag NPs in respect with those of the plasticized PLA mainly for those with maximum at: 868, 895 cm^{-1} (corresponding to the coupling of C–C backbone stretching with the crystalline C–O–C vibration bands), 957 cm^{-1} (O–H vibration of carboxylic acid), 1267 cm^{-1} (C–O–C stretching), 1360, 1383 and 1749 cm^{-1} .

With respect to plasticized PLA film, the increased intensity of bands was observed for ZnO:Cu/Ag 0.5%

Figure 4. EDX image of PLA/ZnO:Cu/Ag 1.5 film

Figure 5. Distribution of elements in PLA loading with different percentage of ZnO:Cu/Ag nanoparticles. a) PLA/ZnO:Cu/Ag 0.5; b) PLA/ZnO:Cu/Ag 1; c) PLA/ZnO:Cu/Ag 1.5

Figure 6. Normalized FTIR spectra of PLA bionanocomposites in comparison with plasticized PLA and neat PLA in the wave numbers range of 2000–750 cm^{-1} . All spectra were recorded on films with thickness below 100 μm .

at 895 and at 957 cm^{-1} which signifies the formation of strong inter-component interactions and a good dispersion of nanoparticles and also that some changes in the polymer chain arrangements occurred [30].

At the same time, the band at 1454 cm^{-1} decreased by 18% for PLA/ZnO:Cu/Ag 0.5% film, by 65% for PLA/ZnO:Cu/Ag 1% film and by 72% in the case of PLA/ZnO:Cu/Ag 1.5%, respectively, in comparison with plasticized PLA film. Also, the incorporation of ZnO:Cu/Ag NPs leads to the decrease of the absorption band at 868 cm^{-1} by 57% for ZnO:Cu/Ag 1%, and by 167% for ZnO:Cu/Ag 1.5% as compared to that of the plasticized PLA. The same spectral behavior, i.e. the increase the maximum intensity of the bands at 1267, 1360 and 1383 cm^{-1} (C–O ester stretch and δCH) for ZnO:Cu/Ag 0.5% but the decrease of their intensity by the incorporation of higher amount of nanoparticles namely for ZnO:Cu/Ag 1% and ZnO:Cu/Ag 1.5% was observed. This could be ex-

plained by changes in crystallinity by nanoparticles incorporation.

3.3. Differential scanning calorimetry (DSC)

It is well-known that PLA under practical processing conditions shows a low crystallinity or it is an amorphous form due to the intrinsic slow crystallization rate, which limits its applications in the automotive and packaging fields. The synergistic effect of plasticizers and nucleating agents in the crystallinity increase was found by the study of the thermal and mechanical properties of PLA nanocomposites [31], in which crystallization by heating (cold crystallization takes place) and crystallinity degree could vary from 12% to more than 30% [5, 32] depending on processing and heating conditions, plasticizer and nucleating agent type and concentration. From the DSC curves of the neat PLA and PLA/ZnO:Cu/Ag bionanocomposites – Figure 7, the thermal characteristics have been determined and they are given in Table 3. The DSC curves of samples displayed a glass transition temperature, a well visible exothermal cold

Figure 7. DSC curves for the neat PLA and its biocomposites with ZnO:Cu/Ag NPs (the second run)

Table 3. Some thermal properties of PLA and PLA bionanocomposites

Sample	T_g [°C]	ΔH_{cc} [J/g]	T_{cc} [°C]	ΔH_m [J/g]	T_m [°C]	χ_c PLA [%]
Neat PLA	57.5	8.3	119.2	9.4	148.7	10.1
PLA/ZnO:Cu/Ag 0	44.0	20.0	95.4	22.9	149.8; 139.2	30.8
PLA/ ZnO:Cu/Ag 0.5	46.4	21.7	98.3	23.1	149.5; 140.2	31.2
PLA/ ZnO:Cu/Ag 1	48.5	20.7	99.1	23.4	149.7; 140.7	31.8
PLA/ ZnO:Cu/Ag 1.5	47.5	21.2	98.7	23.3	149.3; 140.1	31.7

crystallization process and the endothermal melting process as a double peak, probably due to different types of crystalites with different lamellar thickness or reorganization during melting – Figure 7. From DSC curve of neat PLA (Figure 7) is observed a transition at 57.5 °C that is attributed to the glass transition (T_g) relaxational process resulting from micro-Brownian motion of the main chain backbone, a cold crystallization peak at 119.2 °C and a melting peak at 148.7 °C. In comparison with neat PLA, the plasticized blends and bionanocomposites show T_g at lower temperatures. This suggests that the segmental mobility of amorphous pure PLA increases due to the addition of plasticizers [33] and by incorporation of ZnO:Cu/Ag NPs a slight increase in T_g can be noticed in comparison with plasticized PLA (PLA/ZnO:Cu/Ag 0). Plasticized PLA and PLA bionanocomposites exhibit the cold crystallization temperature (T_{cc}) at lower values (95.4–99.1 °C) than neat PLA (119.2 °C), these low values of T_{cc} favor the crystallization process. The increase of ZnO:Cu/Ag NPs content led to shift of T_{cc} at high temperatures (~99.1 °C) than plasticized PLA (95.4 °C). The increase of T_{cc} together with increase of T_g was attributed to the inorganic nanoparticles presence within the PLA matrix [34]. The enthalpy of melting, ΔH_m , increased as the amount of ZnO:Cu/Ag NPs increased in comparison with neat and plasticized PLA – Table 3. Both plasticizer system (ATBC/MB) and ZnO:Cu/Ag nanoparticles act as nucleating agent leading to the increase of the crystallinity degree up to 31.8% with respect to that of the neat PLA and this increase will have an effect on the barrier properties improvements of the PLA bionanocomposites.

3.4. X-ray diffraction results

In Figure 8 the diffraction patterns for ZnO:Cu/Ag nanoparticles and polymer composites embedded with

ZnO:Cu/Ag nanoparticles with Miller indices corresponding to the reflexion peaks are presented. For PLA-composites, the diffraction intensities increase with ZnO:Cu/Ag nanoparticles concentrations. From Figure 8 it can see that the all powder diffraction patterns present a large halo with its maxima at $2\theta = 15.8^\circ$ and a small halo at $2\theta = 31.9^\circ$. Interplanar spacing, d , corresponding to $2\theta = 15.8^\circ$ is 5.6 Å and for $2\theta = 31.9^\circ$ is 2.8 Å. This suggests that the polymer crystalline phase is stratified, the distance between layers being 5.6 Å and the diffraction angles corresponding for the two halos are the order 1 and order 2 of diffraction from Bragg relationship. Two additional diffraction peaks appeared at 38.15° and 44.3° corresponding to Ag metallic nanoparticles with cubic structure [35].

The most frequently occurring interatomic distances, R , is given by Equation (1) [36]:

$$R = 1.25 \cdot d_{\text{Bragg}} \quad (1)$$

Figure 8. X-ray diffraction pattern of the ZnO:Cu/Ag nanoparticles, neat PLA, plasticized PLA and PLA/ZnO:Cu/Ag bionanocomposites films

where d_{Bragg} is the interplanar spacing corresponding to the large halo is $R = 7 \text{ \AA}$, in agreement with literature data [37].

The degree of crystallinity, X_c , was evaluated as the ratio of the diffraction peaks area and the total diffraction area which includes diffraction peaks and amorphous halo [38]. To assess the degree of crystallinity, the Reflex computer program part of Material Studio software suit was used. The values obtained for X_c are summarized in Table 4.

From the data in Table 4 is observed that the incorporation of ZnO:Cu/Ag NPs into PLA and plasticized PLA led to increase of crystallinity of samples, in good correlation with the decreasing of carbonyl band at 1749 cm^{-1} reported in FTIR spectra (Figure 6).

Using the Scherrer formula, the average crystallite sizes for ZnO:Cu–Ag nanoparticles were calculated on the (101), (002) and (100) peaks. The crystallite dimension is 15 nm, in good agreement with the TEM analysis results.

3.5. Transmittance in UV/VIS

One of the desired characteristics of a packaging material is that it must fulfill aesthetic aspect. To determine the light transmission properties of the films, they were scanned at wavelengths ranging from 330 to 800 nm and the percentage light transmittance was recorded (Figure 9).

Film samples exhibited low light transmission in UV light, especially at wavelength of 330 nm. Higher ZnO:Cu/Ag NP content of the bionanocomposites lower percentage UV light transmission is, suggesting a good barrier of biocomposites to ultraviolet light,

Table 4. The crystallinity of PLA, plasticized PLA and PLA/ZnO:Cu/Ag films obtained from XRD analysis

Sample code	X_c [%]
PLA	9.90
PLA/ZnO:Cu/Ag 0%	9.94
PLA/ZnO:Cu/Ag 0.5	11.05
PLA/ZnO:Cu/Ag 1	12.34
PLA/ZnO:Cu/Ag 1.5	13.75

Figure 9. UV light transmission of the PLA, plastified PLA and PLA bionanocomposite films

a powerful lipid-oxidizing agent in food systems. At 375 nm, the PLA/ZnO:Cu/Ag 1% and PLA/ZnO:Cu/Ag 1.5% films show a plasmon resonance characteristic to Ag. In the range of visible spectra, the sample containing 0.5% ZnO:Cu/Ag NPs shows the transmittance around 75%, whereas, in the case of PLA/ZnO:Cu/Ag 1% and PLA/ZnO:Cu/Ag 1.5% films the transmittance values further reduced. This effect is due to the incorporation and distribution of ZnO:Cu/Ag nanoparticles into plasticized PLA as it was observed in Figure 3e–3h. Therefore it can be concluded that the PLA/ZnO:Cu/Ag 0.5% can be used in food packaging materials as a good transparent material.

3.6. Tensile properties

The tensile properties of the PLA-based bionanocomposites are summarized in Table 5.

PLA has a high tensile strength and Young modulus but because of its britleness the elongation at break is very low – Table 5. The addition of plasticizers (MB and ATBC) leads as expected, to an increase of the elongation at break with respect to neat PLA but with a decrease in the tensile strength at break and modulus. The loading of ZnO:Cu/Ag nanoparticles into plasticized PLA has a slowly increased effect of the tensile strength at break with a maximum value of

Table 5. Tensile properties of the neat PLA and PLA/ ZnO:Cu/Ag bionanocomposites

Sample code	Tensile strength at break [MPa]	Elongation at break [%]	Young modulus [MPa]
Neat PLA	69.28±0.18	2.14±0.10	4048±17
PLA/ZnO:Cu/Ag 0	44.81±9.19	3.30±0.64	2898±149
PLA/ ZnO:Cu/Ag 0.5	45.32±7.53	2.78±0.23	2934±161
PLA/ ZnO:Cu/Ag 1	48.39±5.35	2.67±0.52	3058±72
PLA/ ZnO:Cu/Ag 1.5	47.28±2.75	2.61±0.27	3010±107

Table 6. Transmission rate of samples

Sample code	Water vapor [g·m ⁻² ·day ⁻¹]	CO ₂ [mL·m ⁻² ·day ⁻¹]	O ₂ [mL·m ⁻² ·day ⁻¹]
Neat PLA	15.94	873	1308
PLA/ZnO:Cu/Ag 0	13.70	260	104
PLA/ZnO:Cu/Ag 0.5	11.35	230	97
PLA/ZnO:Cu/Ag 1	18.72	Invalid test	Invalid test
PLA/ZnO:Cu/Ag 1.5	15.60	Invalid test	Invalid test

48–47 MPa for ZnO:Cu/Ag 1 or 1.5%. This is possible be due to a homogeneous distribution of ZnO:Cu/Ag nanoparticles into PLA matrix and the high aspect ratio of nanoparticles that restrict the chain movements. A similar improvement in the mechanical properties of PLA film nanocomposites reinforced with both Ag–Cu alloy or ZnO nanoparticles has been reported [23, 30].

3.7. Barrier properties

Water vapor transmittance rate (WVTR) and gas permeability transmittance rate of the developed bionanocomposites are shown in Table 6.

When compared with neat PLA, the incorporation of small amount of ZnO:Cu/Ag NPs (0.5 wt%) into plastified PLA leads to the decrease of the water vapor transmittance rate (WVTR) and gas permeability transmittance rate, therefore the PLA/ZnO:Cu/Ag 0.5 bionanocomposite shows good barrier properties – Table 6. For higher amount of ZnO:Cu/Ag NPs the barrier properties decrease or they can not be evaluated because of inhomogeneity of the films. Considering the reported WVTR of 2.43 g·m⁻²·day⁻¹ and the oxygen transmission rate (OTR) of 2838 mL·m⁻²·day⁻¹ for virgin LDPE film [39] and those of neat PLA reported here, we can appreciate that the film with 0.5% ZnO:Cu/Ag NPs can be considered appropriate for food packaging applications.

3.8. Migration of metals from composites formulations into simulant media

Migration of Zn, Cu, Ag ions from PLA based polymeric formulations destined to food packaging into different simulant media was determined by immersing of biocomposite films into three food simulants [13] for 2 h at 70 °C, as follows: distilled water (simulant A), ethanol 10% (v/v) in aqueous solution (simulant B) and acetic acid 3% (w/v) in aqueous solution (simulant C) – Table 7.

The migration of the Cu and Zn increased with amount of ZnO:Cu/Ag NPs in PLA matrix for all food simulants – Table 7. The Cu ions show a very

Table 7. Metals migration values from bionanocomposites into three food simulants

Sample code	Simulant A	Simulant B	Simulant C
	Zn [mg·dm ⁻²]		
PLA/ZnO:Cu/Ag 0.5	0.248	0.684	0.613
PLA/ZnO:Cu/Ag 1	1.248	3.037	4.280
PLA/ZnO:Cu/Ag 1.5	1.725	3.156	4.550
	Cu [mg·dm ⁻²]		
PLA/ZnO:Cu/Ag 0.5	0.005	0.005	0.040
PLA/ZnO:Cu/Ag 1	0.007	0.019	0.051
PLA/ZnO:Cu/Ag 1.5	0.011	0.185	0.093
	Ag [mg·dm ⁻²]		
PLA/ZnO:Cu/Ag 0.5	0.001	0.001	0.001
PLA/ZnO:Cu/Ag 1	0.001	0.001	0.001
PLA/ZnO:Cu/Ag 1.5	0.001	0.001	0.001

low migration in comparison with Zn ions. Moreover, due to the small quantity of Ag ions in analysed samples, their concentration (0.001 mg·dm⁻²) is close to the detection limit of equipment (0.002 mg·L⁻¹). The overall migration of Zn and Cu ions in acetic acid 3% (w/v) (simulant C) was higher than that occurred in 10% ethanol (simulant B) for all bionanocomposites. However, the overall migration for all samples is below than 10 mg·dm⁻² (the accepted value according to Regulation No 10/2011 [13]).

After the period of the migration test, no visual changes in the surface of films immersed in simulants were observed, and transparency was unaltered. Therefore based on the migration results it can conclude that the PLA-based bionanocomposites are suitable materials for the food packaging.

3.9. Antibacterial activity

Several studies have shown that the bacteria can adhere and form biofilm on different solid materials, such as metal, glass, rubber and plastic. Plastic materials are widely used in the food industry (e.g. as cutting surfaces, packages, tanks, pipes) and medical devices (prosthesis, catheters) where biofilms are undesirable since can cause serious problems such as introducing of continuous infections. Despite efforts to maintain the cleanliness of food packages, a very short contacting time is enough for bacteria to attach

and form biofilms. Therefore, control of the bacterial adhesion plays an important role in maintaining the food quality [40]. In the adherence test, the initial adhesion was measured, which is the interaction between the cell surface and the material surface. The studied PLA-based films modified with ZnO:Cu/Ag nanoparticles were undergone to study their bacterial adherence inhibiting properties against *P. aeruginosa* which is a common Gram-negative bacterium and also against *S. aureus* gram positive bacterium commonly found in soil, water and surfaces of plants. The relative adhesion of different bacterial strains to neat PLA, plasticized PLA and PLA/ZnO:Cu/Ag nanocomposites is shown in Table 8.

The adherence test revealed the high adherence of PLA against *S. aureus* and *P. aeruginosa* test organisms – Table 8. This can be explained by residual lactic acid monomer in commercial PLA. Plasticized PLA showed a high inhibition on *S. aureus*.

The specimens with the highest concentration of ZnO:Cu/Ag NPs showed a better adherence against *S. aureus*, while inhibited the growth of *P. aeruginosa*. The better adhesion to *S. aureus* to polymeric composites is related to electrostatic affinity. Considering that *S. aureus* is a gram positive bacteria, it has more peptidoglycan than gram-negative bacteria because of their thicker cell walls. Thus, since the peptidoglycan is negatively charged and Zn and Cu ions are positively charged in the simulant media, the adherence of gram-positive bacteria is higher than in gram-negative bacteria [41].

Bacterial attachment to inert surfaces is a complicated process being influenced by the properties of both material surface characteristics and bacterial cell. Concerning the material surface characteristics it has been found that the irregularities of surfaces promote bacterial adhesion and biofilm deposition whereas the ultrasmooth surface does not favor bacterial adhesion and biofilm deposition. This may happen since a rough surface has a greater surface area and the depressions in the roughened surfaces provide

more favorable sites for colonization. Also, bacteria adhere and colonize the porous surface preferentially. The important characteristics of bacteria which influence their adherence to a surface are the hydrophobicity and the surface charge [42]. The adhesion process of bacteria to the surfaces include interactions, such as van der Waals, Lewis acid-base, hydrophobic and electrostatic interactions [43]. However the molecular and physical interactions that govern bacterial adhesion to materials have not been understood in detail. Both specific and non-specific interactions may play an important role in the ability of the cell to attach to (or to resist detachment from) the material surface. It can conclude that the ZnO:Cu/Ag NPs incorporation is suitable only for inhibition of the *P. aeruginosa* growth.

4. Conclusions

In this study, the effect of Cu-doped ZnO powder functionalized with AgNPs on the structural, thermal, mechanical and antibacterial properties of the plasticized PLA was investigated.

The crystallinity degree increases with introduction of ZnO:Cu/Ag nanoparticles into plasticized PLA. The water vapor transmittance rate (WVTR) and oxygen transmittance rate (OTR) are improved for PLA/ZnO:Cu/Ag 0.5 in respect with neat PLA. At a high concentration of ZnO:Cu/Ag nanoparticles, their aggregation and non-homogeneous dispersion negatively affect these properties. The overall migration of all samples into three food simulants (distilled water – simulant A, ethanol 10% (v/v) in aqueous solution – simulant B and acetic acid 3% (w/v) in aqueous solution – simulant C was below than $10 \text{ mg} \cdot \text{dm}^{-2}$, accepted value according to EU Regulation No 10/2011 for plastic materials and articles intended to come into contact with food. Also, the PLA nanocomposites inhibited the growth of *P. aeruginosa* but they were not efficient as antimicrobial packaging against *S. aureus*.

Based on the obtained results it can conclude that the PLA/ZnO:Cu/Ag 0.5 bionanocomposite shows the best properties which offers suitable mechanical and thermal properties, good barrier properties to ultraviolet light, water vapour, oxygen and carbon dioxide, antibacterial activity and low migration of nanoparticles into food simulants. It can be appreciated that the new developed PLA/ZnO:Cu/Ag bionanocomposites are promising materials for food packaging application.

Table 8. The relative adhesion of different bacterial strains to neat PLA and PLA/ZnO:Cu/Ag samples

Sample	<i>S. aureus</i> [CFU/cm ²]	<i>P. aeruginosa</i> [CFU/cm ²]
PLA	$2.8 \pm 0.1 \cdot 10^3$	$2.3 \pm 0.4 \cdot 10^4$
PLA/ZnO:Cu/Ag 0	$1.0 \pm 0.2 \cdot 10^3$	$1.9 \pm 0.2 \cdot 10^4$
PLA/ZnO:Cu/Ag 0.5	$3.3 \pm 0.1 \cdot 10^3$	$4.0 \pm 0.1 \cdot 10^3$
PLA/ZnO:Cu/Ag 1	$6.5 \pm 0.1 \cdot 10^3$	$6.3 \pm 0.1 \cdot 10^2$
PLA/ZnO:Cu/Ag 1.5	$1.0 \pm 0.1 \cdot 10^4$	$2.05 \pm 0.2 \cdot 10^2$

Acknowledgements

The research leading to these results has received funding from the Romanian ANCSI, Project ID P_40_404, under the Contract No 18/01.09.2016.

References

- [1] Rhim J-W., Park H-M., Ha C-S.: Bio-nanocomposites for food packaging applications. *Progress in Polymer Science*, **38**, 1629–1652 (2013).
<https://doi.org/10.1016/j.progpolymsci.2013.05.008>
- [2] Huang J-W., Hung Y. C., Wen Y-L., Kang C-C., Yeh M-Y.: Polylactide/nano and microscale silica composite films. I. Preparation and characterization. *Journal of Applied Polymer Science*, **112**, 1688–1694 (2009).
<https://doi.org/10.1002/app.29616>
- [3] Lagaron J. M., Lopez-Rubio A.: Nanotechnology for bioplastics: Opportunities, challenges and strategies. *Trends in Food Science and Technology*, **22**, 611–617 (2011).
<https://doi.org/10.1016/j.tifs.2011.01.007>
- [4] Sánchez Aldana D., Duarte Villa E., De Dios Hernández M., González Sánchez G., Rascón Cruz Q., Flores Gallardo S., Piñon Castillo H., Ballinas Casarrubias L.: Barrier properties of polylactic acid in cellulose based packages using montmorillonite as filler. *Polymers*, **6**, 2386–2403 (2014).
<https://doi.org/10.3390/polym6092386>
- [5] Răpă M., Miteluț A. C., Tănase E. E., Grosu E., Popescu P., Popa M. E., Rosnes J. T., Sivertsvik M., Darie-Niță R. N., Vasile C.: Influence of chitosan on mechanical, thermal, barrier and antimicrobial properties of PLA-biocomposites for food packaging. *Composites Part B: Engineering*, **102**, 112–121 (2016).
<https://doi.org/10.1016/j.compositesb.2016.07.016>
- [6] Li Y., Han C., Bian J., Han L., Dong L., Gao G.: Rheology and biodegradation of polylactide/silica nanocomposites. *Polymer Composites*, **33**, 1719–1727 (2012).
<https://doi.org/10.1002/pc.22306>
- [7] Sanchez-Garcia M. D., Lopez-Rubio A., Lagaron J. M.: Natural micro and nanobiocomposites with enhanced barrier properties and novel functionalities for food biopackaging applications. *Trends in Food Science and Technology*, **21**, 528–536 (2010).
<https://doi.org/10.1016/j.tifs.2010.07.008>
- [8] Chen C-C., Chueh J-Y., Tseng H., Huang H-M., Lee S-Y.: Preparation and characterization of biodegradable PLA polymeric blends. *Biomaterials*, **24**, 1167–1173 (2003).
[https://doi.org/10.1016/S0142-9612\(02\)00466-0](https://doi.org/10.1016/S0142-9612(02)00466-0)
- [9] Tri P. N., Domenek S., Guinault A., Sollogoub C.: Crystallization behavior of poly(lactide)/poly(β -hydroxybutyrate)/talc composites. *Journal of Applied Polymer Science*, **129**, 3355–3365 (2013).
<https://doi.org/10.1002/app.39056>
- [10] Battagazzore D., Bocchini S., Frache A.: Crystallization kinetics of poly(lactic acid)-talc composites. *Express Polymer Letters*, **5**, 849–858 (2011).
<https://doi.org/10.3144/expresspolymlett.2011.84>
- [11] Armentano I., Fortunati E., Burgos N., Dominici F., Luzi F., Fiori S., Jiménez A., Yoon K., Ahn J., Kang S., Kenny J. M.: Processing and characterization of plasticized PLA/PHB blends for biodegradable multiphase systems. *Express Polymer Letters*, **9**, 583–596 (2015).
<https://doi.org/10.3144/expresspolymlett.2015.55>
- [12] Arrieta M. P., López J., Hernández A., Rayón E.: Ternary PLA–PHB–Limonene blends intended for biodegradable food packaging applications. *European Polymer Journal*, **50**, 255–270 (2014).
<https://doi.org/10.1016/j.eurpolymj.2013.11.009>
- [13] Union Guidelines on Regulation (EU) N 10/2011 On plastic materials and articles intended to come into contact with food. Official Journal of European Communities (2011).
- [14] Vasile C.: Polymeric materials for food packaging. in ‘New polymeric food packagings’ (eds.: Vasile C., Cheaburu C. N). PIM, Iasi, 9–57 (2010).
- [15] Silvestre C., Duraccio D., Cimmino S.: Food packaging based on polymer nanomaterials. *Progress in Polymer Science*, **36**, 1766–1782 (2011).
<https://doi.org/10.1016/j.progpolymsci.2011.02.003>
- [16] Espitia P. J. P., de Fátima Ferreira Soares N., dos Reis Coimbra J. S., de Andrade N. J., Cruz R. S., Medeiros E. A. A.: Zinc oxide nanoparticles: Synthesis, antimicrobial activity and food packaging applications. *Food and Bioprocess Technology*, **5**, 1447–1464 (2012).
<https://doi.org/10.1007/s11947-012-0797-6>
- [17] Dhapte V., Gaikwad N., More P. V., Banerjee S., Dhapte V. V., Kadam S., Khanna P. K.: Transparent ZnO/poly-carbonate nanocomposite for food packaging application. *Nanocomposites*, **1**, 106–112 (2015).
<https://doi.org/10.1179/2055033215Y.0000000004>
- [18] Tankhiwale R., Bajpai S. K.: Preparation, characterization and antibacterial applications of ZnO-nanoparticles coated polyethylene films for food packaging. *Colloids and Surfaces B: Biointerfaces*, **90**, 16–20 (2012).
<https://doi.org/10.1016/j.colsurfb.2011.09.031>
- [19] Silvestre C., Duraccio D., Marra A., Strongone V., Cimmino S.: Development of antibacterial composite films based on isotactic polypropylene and coated ZnO particles for active food packaging. *Coatings*, **6**, 4/1–4/14 (2016).
<https://doi.org/10.3390/coatings6010004>
- [20] Cushen M., Kerry J. P., Morris M., Cruz-Romero M., Cummins E.: Evaluation and simulation of silver and copper nanoparticle migration from polyethylene nanocomposites to food and an associated exposure assessment. *Journal of Agricultural and Food Chemistry*, **62**, 1403–1411 (2014).
<https://doi.org/10.1021/jf404038y>
- [21] de Moura M. R., Mattoso L. H. C., Zucolotto V.: Development of cellulose-based bactericidal nanocomposites containing silver nanoparticles and their use as active food packaging. *Journal of Food Engineering*, **109**, 520–524 (2012).
<https://doi.org/10.1016/j.jfoodeng.2011.10.030>

- [22] Longano D., Ditaranto N., Cioffi N., Di Niso F., Sibillano T., Ancona A., Conte A., Del Nobile M. A., Sabbatini L., Torsi L.: Analytical characterization of laser-generated copper nanoparticles for antibacterial composite food packaging. *Analytical and Bioanalytical Chemistry*, **403**, 1179–1186 (2012).
<https://doi.org/10.1007/s00216-011-5689-5>
- [23] Ahmed J., Arfat Y. A., Castro-Aguirre E., Auras R.: Mechanical, structural and thermal properties of Ag–Cu and ZnO reinforced polylactide nanocomposite films. *International Journal of Biological Macromolecules*, **86**, 885–892 (2016).
<https://doi.org/10.1016/j.ijbiomac.2016.02.034>
- [24] Ahmed J., Arfat Y. A., Castro-Aguirre E., Auras R.: Thermal properties of ZnO and bimetallic Ag–Cu alloy reinforced poly(lactic acid) nanocomposite films. *Journal of Thermal Analysis and Calorimetry*, **125**, 205–214 (2016).
<https://doi.org/10.1007/s10973-016-5402-1>
- [25] Brihi N., Bouaine A., Berbadj A., Schmerber G., Colis S., Dinia A.: No ferromagnetic properties in polycrystalline Al-doped $\text{Zn}_{0.97}\text{Mn}_{0.03}\text{O}$ diluted magnetic semiconductor. *Thin Solid Films*, **518**, 4549–4552 (2010).
<https://doi.org/10.1016/j.tsf.2009.12.028>
- [26] Vivekanandhan S., Schreiber M., Mason C., Mohanty A. K., Misra M.: Maple leaf (*Acer* sp.) extract mediated green process for the functionalization of ZnO powders with silver nanoparticles. *Colloids and Surfaces B: Biointerfaces*, **113**, 169–175 (2014).
<https://doi.org/10.1016/j.colsurfb.2013.08.033>
- [27] Auras R. A., Singh S. P., Singh J. J.: Evaluation of oriented poly(lactide) polymers vs. existing PET and oriented PS for fresh food service containers. *Packaging Technology and Science*, **18**, 207–216 (2005).
<https://doi.org/10.1002/pts.692>
- [28] Lazar M. A., Vodnar D., Prodan D., Rotaru H., Roman C. R., Sorcoi L. A., Baciut G., Campian R. S.: Antibacterial coating on biocomposites for cranio-facial reconstruction. *Clujul Medical*, **89**, 430–434 (2016).
<https://doi.org/10.15386/cjmed-599>
- [29] Dorigato A., Sebastiani M., Pegoretti A., Fambri L.: Effect of silica nanoparticles on the mechanical performances of poly(lactic acid). *Journal of Polymers and the Environment*, **20**, 713–725 (2012).
<https://doi.org/10.1007/s10924-012-0425-6>
- [30] Jayaramudu J., Das K., Sonakshi M., Reddy G. S. M., Aderibigbe B., Sadiku R., Ray S. S.: Structure and properties of highly toughened biodegradable polylactide/ZnO biocomposite films. *International Journal of Biological Macromolecules*, **64**, 428–434 (2014).
<https://doi.org/10.1016/j.ijbiomac.2013.12.034>
- [31] Lim L-T., Auras R., Rubino M.: Processing technologies for poly(lactic acid). *Progress in Polymer Science*, **33**, 820–852 (2008).
<https://doi.org/10.1016/j.progpolymsci.2008.05.004>
- [32] Battezzatore D., Bocchini S., Frache A.: Crystallization kinetics of poly(lactic acid)-talc composites. *Express Polymer Letters*, **5**, 849–858 (2011).
<https://doi.org/10.3144/expresspolymlett.2011.84>
- [33] Darie-Niță R. N., Vasile C., Irimia A., Lipșa R., Râpă M.: Evaluation of some eco-friendly plasticizers for PLA films processing. *Journal of Applied Polymer Science*, **133**, 43223/1–43223/11 (2016).
<https://doi.org/10.1002/app.43223>
- [34] Liu X., Wang T., Chow L. C., Yang M., Mitchell J. W.: Effects of inorganic fillers on the thermal and mechanical properties of poly(lactic acid). *International Journal of Polymer Science*, **2014**, 827028/1–827028/8 (2014).
<https://doi.org/10.1155/2014/827028>
- [35] Wan X., Liang X., Zhang C., Li X., Liang W., Xu H., Lan S., Tie S.: Morphology controlled syntheses of Cu-doped ZnO, tubular Zn(Cu)O and Ag decorated tubular Zn(Cu)O microcrystals for photocatalysis. *Chemical Engineering Journal*, **272**, 58–68 (2015).
<https://doi.org/10.1016/j.cej.2015.02.089>
- [36] Klug H. P., Alexander L. E.: X-ray diffraction procedure for polycrystalline and amorphous materials. Wiley, New York (1974).
- [37] Lu Y., Chen Y-C., Zhang P-H.: Preparation and characterisation of polylactic acid (PLA)/polycaprolactone (PCL) composite microfibre membranes. *Fibres and Textiles in Eastern Europe*, **24**, 17–25 (2016).
<https://doi.org/10.5604/12303666.1196607>
- [38] Pascuta P., Vladescu A., Borodi G., Culea E., Tetea R.: Structural and magnetic properties of zinc ferrite incorporated in amorphous matrix. *Ceramics International*, **37**, 3343–3349 (2011).
<https://doi.org/10.1016/j.ceramint.2011.05.134>
- [39] Reesha K. V., Panda S. K., Bindu J., Varghese T. O.: Development and characterization of an LDPE/chitosan composite antimicrobial film for chilled fish storage. *International Journal of Biological Macromolecules*, **79**, 934–942 (2015).
<https://doi.org/10.1016/j.ijbiomac.2015.06.016>
- [40] Bayoumi M. A., Kamal R. M., Abd El Aal S. F., Awad E. I.: Assessment of a regulatory sanitization process in Egyptian dairy plants in regard to the adherence of some food-borne pathogens and their biofilms. *International Journal of Food Microbiology*, **158**, 225–231 (2012).
<https://doi.org/10.1016/j.ijfoodmicro.2012.07.021>
- [41] Magyari K., Stefan R., Vodnar D. C., Vulpoi L.: The silver influence on the structure and antibacterial properties of the bioactive $10\text{B}_2\text{O}_3\text{--}30\text{Na}_2\text{O--}60\text{P}_2\text{O}_5$ glass. *Journal of Non-Crystalline Solids*, **402**, 182–186 (2014).
<https://doi.org/10.1016/j.jnoncrystol.2014.05.033>
- [42] Katsikogianni M., Missirlis Y. F.: Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteriamaterial interactions. *European Cells and Materials*, **8**, 37–57 (2004).
<https://doi.org/10.22203/eCM.v008a05>
- [43] Faille C., Jullien C., Fontaine F., Bellon-Fontaine M-N., Slomiany C., Benezech T.: Adhesion of *Bacillus* spores and *Escherichia coli* cells to inert surfaces: Role of surface hydrophobicity. *Canadian Journal of Microbiology*, **48**, 728–738 (2002).
<https://doi.org/10.1139/w02-063>