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PLA/PVP/bio-synthesized hydrozincite nanocomposite films – photocatalytic and antibacterial activity

K. L. Zaharieva[1*], S. S. Dimova[2], R. T. Eneva[3], V. N. Hubenov[3], I. D. Stambolova[4], D. D. Stoyanova[4], F. S. Ublekov[2], O. S. Dimitrov[5], H. P. Penchev[2]

[1]*Institute of Mineralogy and Crystallography "Acad. I. Kostov", Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 107, 1113 Sofia, Bulgaria*
[2]*Institute of Polymers, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 103A, 1113 Sofia, Bulgaria*
[3]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 26, 1113 Sofia, Bulgaria*
[4]*Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 11, 1113 Sofia, Bulgaria*
[5]*Institute of Electrochemistry and Energy Systems, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 10, 1113 Sofia, Bulgaria*

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Nanocomposite films based on biodegradable polylactic acid (PLA) and PLA/polyvinylpyrrolidone (PVP) with embedded stabilized Hydrozincite powder were synthesized by solution casting method. The Hydrozincite was prepared by plant extract-mediated hydrothermal synthesis. Powder X-ray diffraction analysis and Fourier-transform infrared spectroscopy were used to establish the phase composition and structure of the films. The photocatalytic activity of the obtained nanocomposite films was tested and compared in the reaction of degradation of Malachite Green dye under UV illumination. The photocatalytic results revealed that the PLA/PVP-containing Hydrozincite nanocomposite film leads to a higher degree of degradation of the Malachite Green dye (87 %) after 150 minutes in comparison to that of PLA/Hydrozincite (31%). The antibacterial activity of the prepared nanocomposite films against an *Escherichia coli* control strain was also discussed. The investigated biocomposite films demonstrated high antibacterial efficiency. It was found that the concentration of viable bacterial cells decreases by about 99% after 1 hour of contact time for both investigated composites.

Keywords: PLA/PVP/Hydrozincite, nanocomposite, photocatalyst, Malachite Green, antibacterial activity*:*

INTRODUCTION

The polymeric/inorganic nanocomposites have a wide variety of applications in photocatalysis, engineering technology and medicine, due to their suitable electronic, optical and mechanical properties [1-3]. Polylactide (PLA) is an environmentally friendly, economical and commercially available polymer that can be used as disposable packaging material [4]. PVP is one of the most widely used vinyl polymers possessing very interesting properties, such as: extremely low cytotoxicity, high chemical and thermal resistance, bio- and hemocompatibility, good environmental stability [5]. Hydrozincite ($Zn_5(CO_3)_2(OH)_6$) is a known carbonate mineral of zinc which can be formed synthetically or naturally (geologically and biologically) [6]. Organic contaminants such as dyes released from textile, pharmaceutical and other industries have caused serious water pollution problems. Previous investigations have revealed that the photocatalytic degradation process is one of the effective, low-cost and green solutions to resolve these problems [7]. The photocatalytic properties and/or antibacterial efficiency of various hybrid composites such as PLA/ZnO, PLA/ZnO/TiO_2, PLA/ZnO:Cu/Ag, PVP/ZnO, Cd/Ag/ZnO/PVP, PVP/SnO_2/TiO_2 have been discussed in the literature [8-15].

The development of novel polymers and polymer-based materials with antibacterial properties is usefull in many different applications, such as biomedicine (drug delivery systems), food science and technology, etc. [16]. One of the strategies to enhance the antimicrobial properties of composite polymers is the incorporation of non-toxic oxide nanoparticles (e.g. ZnO, TiO_2) in the polymer matrix. Biocompatible polymers filled with antibacterial particles (Ag, Cu, TiO_2, ZnO) are preferred; for instance, those based on polyhydroxyalkanoates such as polyhydroxy-butyrate (PHB) and biodegradable polyesters like polylactic acid (PLA) and polyglycolic acid (PGA) [17].

* To whom all correspondence should be sent:
E-mail: *zaharieva@imc.bas.bg*

The aim of this study is to prepare nanocomposite films based on polylactic acid (PLA) and PLA/polyvinylpyrrolidone (PVP) matrix, containing Hydrozincite green synthesized particles. The phase composition and structure were studied using powder X-ray diffraction analysis and Fourier-transform infrared spectroscopy. The photocatalytic behavior of the obtained hybrid materials in the degradation reaction of Malachite Green (MG) dye under UV light and their antibacterial properties against the pathogen *Escherichia coli* were investigated.

EXPERIMENTAL

Materials

The polymers, poly(D,L-lactide) (PLA), M_w = 60.520 g.mol^{-1}, Shenzhen Esun Industrial Co., Ltd. and polyvinylpyrrolidone (PVP), M_w = 360.000, Fluka AG, dichloromethane (Valerus Co.), ethanol (96 % Fisher Scientific, U.K). All chemicals were of analytical grade and were used as received without any further purification.

Preparation of PLA/Hydrozincite and PLA/PVP/Hydrozincite films. Films of nanocomposite blend (PLA-PVP) with incorporated hydrozincite (5 wt.%) were prepared by a solution casting method (Figure 1). For the film preparation, PLA stock solution was prepared by dissolving 1 g of PLA in 30 mL of dichloromethane (DCM). Hydrozincite nanoparticles (5 wt.%) prepared using *Mentha arvensis* plant extract-mediated hydrothermal synthesis [18], were well dispersed in 10 mL of DCM by magnetic stirring for 30 min at room temperature followed by sonication (frequency, 20 kHz; power, 200 W).

For PLA/Hydrozincite nanocomposite film preparation, these two solutions were mixed together with magnetic stirring.

For PLA/PVP/Hydrozincite film preparation, a stock solution of PVP, 0.1g in 10 mL ethanol was prepared and mixed with the Hydrozincite suspension in DCM. In the next step, the solution of PLA and the suspension of PVP-stabilized Hydrozincite were vigorously mixed by vortex. Finally, both PLA/Hydrozincite and PLA/PVP-Hydrozincite dispersions were sonicated for 30 min until a homogenized blend was obtained. The resulting solutions were poured into a Teflon mold (8×4×1.5 cm) and rested under hood till the solvent was completely evaporated, resulting a dry 60 μm thick film.

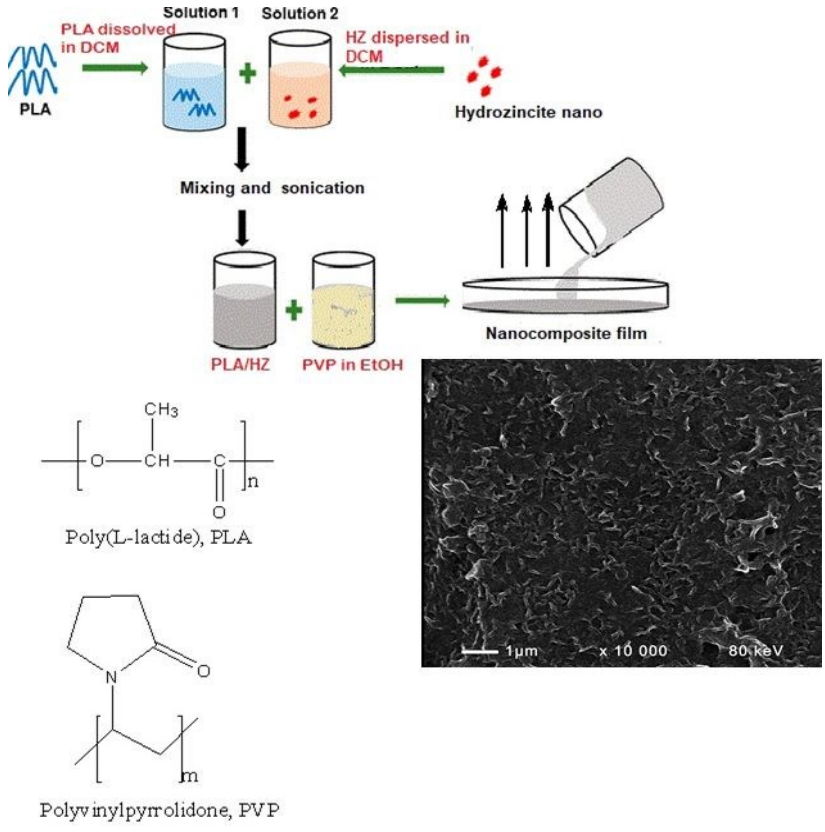

Fig. 1. Preparation of the PLA/PVP/Hydrozincite nanocomposite films

Physicochemical characterization

The physicochemical characterization of the samples was performed using FT-IR spectroscopy and powder X-ray diffraction analysis (PXRD). PXRD analysis was carried out on a Bruker D8 Advance ECO diffractometer in reflection mode with Ni-filtered Cu Kα radiation at 40 kV. The FT-IR spectroscopy was carried out on a Fourier infrared spectrometer Bruker-Vector 22 in the range 400-4000 cm^{-1}. The FT-IR spectra of the samples were registered using KBr tablets.

Photocatalytic tests

The oxidative photodegradation of an aqueous solution of Malachite Green dye with concentration of 5 ppm was investigated in a semi-batch slurry reactor using the synthesized PLA/Hydrozincite and PLA/PVP/Hydrozincite films as catalyst and 60 ml of dye solution under constant stirring in an air flow. The photocatalytic tests were carried out using UV-Vis absorbance spectrophotometer in the wavelength range from 200 to 800 nm (λ_{max} = 615 nm) and polychromatic UV-A lamp illumination (18W) with maximum of the emission at 365 nm and intensity of illumination 0.66 $mW.cm^{-2}$. The tested systems were left in the dark for about 30 min before switching on the UV irradiation for 2 hours and 30 minutes in order to reach adsorption-desorption equilibrium state. Periodically sample aliquots were taken from the solution. The degree of dye degradation was calculated using the dependence: $((C_0\text{-}C)/C_0) \times 100$, where Co and C are initial concentration before turning on the illumination and residual concentration of the dye solution after illumination for a selected time interval.

Antibacterial ability

The antimicrobial activity was determined by placing an appropriate amount of the polymer sample in contact with a microbial suspension of a certain cell density. A modification of the ASTM standard test method E 2149-10 was used, which consisted of placing a 0.1 g sample of the composite film in contact with 1 ml of a microbial suspension of *E. coli* (ATCC 25922) in a well of a 24-well test plate (Techno Plastic Products AG, Switzerland). During cultivation, the plate was placed on an Advantage-Lab shaker apparatus, AL05-06, which provided continuous agitation at 150 rpm. Cultivation was carried out at a constant temperature of 20 ± 1°C provided in a thermostated room. The antimicrobial effect was determined by counting the number of colony-forming units (CFU) in aliquots of the microbial suspension that was in contact with the composite film at certain time intervals after the start of cultivation (1 h, 24 h, 48 h and on the fifth day).

The bacterial suspension used in the experiment was prepared from an 18-hour shake culture of *E. coli* (ATCC 25922). The composition of the nutrient medium used for the development of the strain is as follows (for 1 L): 10 g of tryptone, 5 g of yeast extract and 10 g of sodium chloride. The 18 h culture grown in the indicated medium was centrifuged at 3500 rpm/10 min. The collected cell pellets were washed and resuspended in a sterile buffer with the following composition (for 1 liter): 0.150g KCl, 2.25g NaCl, 0.05g $NaHCO_3$, 0.12g $CaCl_2.6H_2O$ (pH 7.0). The final concentration of the bacterial cell suspension should be in the range of $1.5\text{-}3.0\times10^6$ CFU [19].

RESULTS AND DISCUSSION

The PXRD patterns of the investigated samples are presented on Figure 2.

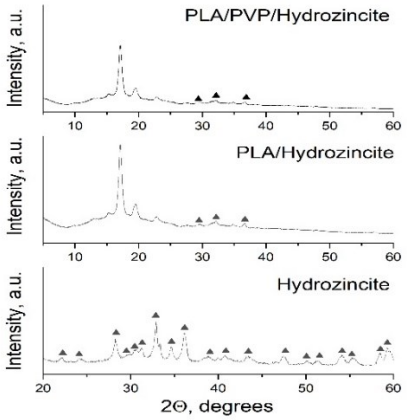

Fig. 2. PXRD patterns of PLA/Hydrozincite, PLA/PVP/Hydrozincite films and Hydrozincite.

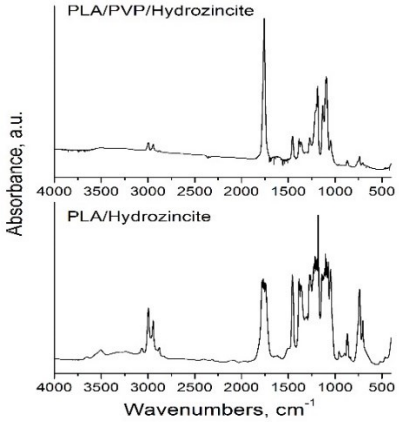

Fig. 3. FT-IR spectra of PLA//Hydrozincite and PLA/PVP/Hydrozincite films.

The PXRD analyses of PLA/Hydrozincite and PLA/PVP/Hydrozincite films established diffraction peaks, corresponding to an orthorhombic crystal structure of PLA. The PXRD patterns of PLA/Hydrozincite and PLA/PVP/Hydrozincite films showed additional three diffraction peaks, corresponding to hydrozincite phase (JCPDS 72-1100) at 29.02°, 33.06° and 36.95° 2Θ. Due to the amorphous nature of PVP and its small quantity, its presence was not registered.

Figure 3 represents the FT-IR spectra of PLA/Hydrozincite and PLA/PVP/Hydrozincite films obtained in the range of 400-4000 cm^{-1} at room temperature. Characteristic absorption peaks were found around 1750 cm^{-1} which correspond to the stretching vibration of -C=O and a peak at 1260 cm^{-1}, which was attributed to the bending vibration of the -CH(CH_3) plane. The bending vibration at 1090 cm^{-1} is due to the presence of ester groups -C-O, which belong to PLA [20]. The absorption peak at 2883 cm^{-1} is due to the C−H stretching vibrations and the peak registered at 1346 cm^{-1} corresponds to the C−O−H bending vibrations. The peak at 1180 cm^{-1} could be attributed to the C−O-C stretching vibrations. The peaks around 2900-2850 cm^{-1} are due to the C-H stretching vibrations and the peak observed at 1400 cm^{-1} corresponds to the PVP [21]. The absorption bands at 830, 1383, 1510 cm^{-1} are assigned to the characteristic vibrations of hydrozincite [22].

The photocatalytic activities of the prepared PLA/Hydrozincite and PLA/PVP/Hydrozincite nanocomposite films were tested and compared in the reaction of degradation of MG dye as model pollutant in aqueous solutions under UV irradiation. Figures 4A and 4B present the concentration changes C/C_0 and the degree of degradation of MG dye with time of UV illumination, respectively. The photocatalytic results revealed that PLA/PVP/Hydrozincite nanocomposite photocatalysts demonstrate higher photocatalytic activity in MG dye degradation (87%) in comparison to that of PLA/Hydrozincite films (31%). The values of apparent rate constants (calculated using logarithmic linear dependence:–ln(C/C_0) = k.t.) of the studied PLA/PVP/Hydrozincite and PLA/Hydrozincite catalysts are $12.3\times10^{-3}min^{-1}$ and $2.7\times10^{-3}min^{-1}$, respectively. It could be supposed that the PVP surface stabilized Hydrozincite and the improved PVP/Hydrozincite dispersibility into the PLA matrix plays a significant role for the enhanced photocatalytic activity of these composite films. Other research groups have also established an improvement of the catalytic activity towards degradation of both sulfamethoxazole and Reactive Red-141 dye in the presence of PVP-surface stabilized ZnO [23, 24].

The antimicrobial properties of the investigated materials were evaluated with *E. coli* (ATCC 25922) strain. A standardized suspension of *E. coli* strain was used as a control and PLA foil immersed in buffer solution was used as a blank and was found to contain $5.9.10^6$ CFU/mL. Both composites exhibit high antimicrobial activity against *E. coli* and above 99 % of reduction in CFU number since the first sampling after 1 hour of cultivation of the bacterial suspension in contact with the material (Table 1).

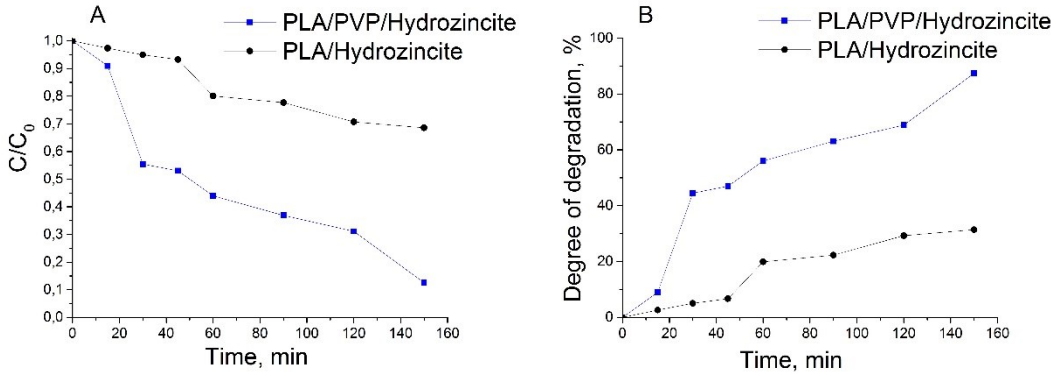

Fig. 4. A) The concentration ratio C/C_0 and **B)** Degree of degradation of Malachite Green dye with time of UV illumination**.**

The antibacterial efficiency of the obtained hybrid films might be due to the release of zinc ions from the hydrozincite particles, which can pass through the cell membrane, causing oxidative damage. These processes might be responsible for the enhanced antibacterial activity of the PLA/PVP/Hydrozincite and PLA/Hydrozincite nanocomposite films [25, 26].

Table 1. Log reduction of colony-forming units (CFU) in *E. coli* K-12 suspension placed in contact with different biopolymer composites.

Cultivation time, h	Control (*E. coli*) Log Reduction	Biocomposite samples	
		PLA/hydrozincite Log Reduction	PLA/PVP/hydrozincite Log Reduction
0	0	0	0
1	0	4.071	3.771
24	0	4.771	4.771
48	0	4.771	4.771
120	0	4.771	4.771

CONCLUSIONS

A solution casting method was successfully used to prepare PLA/Hydrozincite and PLA/PVP/ Hydrozincite films. The PLA/PVP/Hydrozincite hybrid photocatalysts demonstrated enhanced photocatalytic activity towards degradation of MG dye (87%) after 150 minutes of UV irradiation. The obtained composite PLA/PVP/Hydrozincite could be applied for purification of water polluted with Malachite Green textile dye. Both PLA and PLA/PVP-embedded bio-synthesized hydrozincite composites have excellent bactericidal activity against *E. coli* (90% after 1 hour of contact time). The prepared polymer/inorganic nanocomposite films are promising candidates as alternative materials in various food packing applications.

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