Journal of composites science

MDPI

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Innovative Composites Based on Organic Modified Zirconium Phosphate and PEOT/PBT Copolymer

Maria Bastianini [1,*], Marco Scatto [2], Michele Sisani [1], Paolo Scopece [2], Alessandro Patelli [3] and Annarita Petracci [1]

1 Prolabin & Tefarm S.r.l., Via dell'Acciaio 9, 06134 Perugia, Italy; michele.sisani@prolabintefarm.com (M.S.); annarita.petracci@prolabintefarm.com (A.P.)
2 Nadir S.r.l., c/o Scientific Campus University Ca' Foscari Venezia, Via Torino 155b, 30172 Mestre, Italy; scatto@nadir-tech.it (M.S.); scopece@nadir-tech.it (P.S.)
3 Department of Physics and Astronomy, Padova University, via Marzolo 8, 35122 Padova, Italy; alessandro.patelli@unipd.it
* Correspondence: maria.bastianini@prolabintefarm.com; Tel.: +39-328-714-2344

Received: 4 April 2018; Accepted: 15 May 2018; Published: 17 May 2018

Abstract: Polymers are key building blocks in the development of smart materials for biomedical applications, and many polymers offer unique properties for specific applications. A wide range of materials is available through the use of polymer compounds. These compounds can incorporate performance-enhancing fillers, which provide properties not reachable with ordinary neat polymers (e.g., bending stiffness, tensile strength, elongation, torque, biological activity such as antimicrobial properties, cell differentiation). In this work, the preparation of functional biocomposites containing organic modified zirconium phosphate (ZrP) as drug carrier is presented. The composites were prepared by melt compounding, which offers significant promise since it allows an easy customization of the plastic compounds that well suit biomedical applications (devices, long-term implantable polymers, bioresorbable polymers). The obtained polymer composites based on ZrP intercalated with gentamicin (GMT) and poly(ethylene oxide terephthalate)/poly(butylene terephthalate) (PEOT/PBT) were characterized.

Keywords: zirconium phosphate; gentamicin; polymer composite; melt compounding; antimicrobial activity

1. Introduction

Organic–inorganic hybrid materials based on lamellar solids are innovative compounds that allow the development of multifunctional fillers for polymer composites [1,2]. Alpha-Zirconium phosphate is a lamellar solid with interesting properties to be used for the development of such hybrid materials [3].

ZrP structure indeed is composed by stacking layers made of planes of Zr atoms bonded, on both sides, to monohydrogen phosphate groups. Each phosphate group is bonded to three Zr atoms of the plane, while each zirconium is octahedrally coordinated by six oxygens of six different monohydrogen phosphate groups. The water molecules are located in the zeolitic cavities of the interlayer region [4]. Zirconium phosphate can be easily functionalized through intercalation reactions, and has been widely studied in academic literature with the aim of vehiculate bioactive organic molecules [5–9]. The term 'intercalation' refers to a reversible process where a guest molecule or ion is inserted into the interlayer region of a host lamellar matrix [10]. In Figure 1, it is reported a scheme of the intercalation process, where the host is Zirconium Phosphate and the guest is the organic active. Moreover, the electrostatic interactions [11] of the guest molecules and the lamellae of the ZrP also increase its chemical and thermal stability.

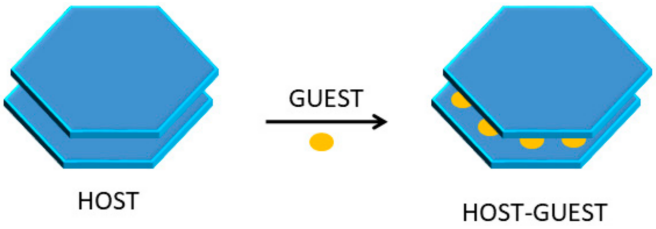

Figure 1. Scheme of an intercalation reaction.

Lamellar systems can be used as fillers to produce polymer composites with antimicrobial activity [12–16]. Donnadio et al. described the use of ZrP modified with chlorhexidine to confer antimicrobial properties to carboxymethylcellulose films [17].

Since the production of polymeric compounds is a tailor made activity, mainly in the field biomedical application [18], the melt compounding approach for the composites preparation is a promising and suitable solution because of its low cost, high productivity and compatibility with the current polymer processing techniques [19,20]. In this work, the preparation and characterization of an organic-inorganic hybrid based on gentamicin antibiotic and synthetic zirconium phosphate (ZrP-GTM) are presented [21]. This hybrid was employed as filler for the preparation of the polymer composites produced with the melt compounding technology assisted by twin screw extruder.

For this purpose, the poly(ethylene oxide terephthalate)/poly(butylene terephthalate) copolymer (PEOT/PBT) was chosen. The thermoplastic elastomeric properties of these multi-block copolymers are obtained by phase separation of the hydrophilic and hydrophobic segments in the polymers; this makes this block of copolymers highly interesting for the 3D printing manufacturing [22,23]. The polymer has a relatively low melting viscosity, while after cooling it exhibits very good mechanical properties. The possibility to balance hydrophobicity/hydrophilicity of the surface also makes these polymers very interesting for tissue engineering [24].

To the best of our knowledge, many works have been published on the preparation of composites between thermoplastic polymers and modified or non-modified zirconium phosphate by melt-compounding extrusion [18,20,25–29], but no one has reported the preparation of a ZrP-GTM and PBT/PEOT composite with a twin screw extruder. Polymeric compounds containing increasing amount of filler were chemical-physically and biologically characterized.

2. Materials and Methods

2.1. Chemicals and Solvent

Propylamine was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). Zirconyl chloride octahydrate was supplied by MEL Chemicals (Manchester, United Kingdom) and phosphoric acid 75% was purchased from C.M. Chimica (Ponte Buggianese, Italy). Gentamicin sulfate (GTM) was supplied by Chemical Point (Milan, Italy). Poly(ethylene oxide)/poly(butylene terephthalate) 300PEOT55PBT45 copolymer, labelled as PBT/PEOT, was provided by PolyVations BV (Groningen, Netherlands). The solvents and materials were of reagent grade and used without further purification.

2.2. ZrP Synthesis

Crystalline zirconium phosphate $Zr(HPO_4)_2 \cdot H_2O$ was obtained by refluxing zirconyl chloride ($ZrOCl_2 \cdot 8H_2O$) in a 10 M phosphoric acid solution for 48 h [30]. The residual solid was centrifuged, washed 3 times with water and dried in oven at 60 °C.

2.3. Intercalation of GTM in α-ZrP

A pre-intercalated phase of α-ZrP with an expanded interlayer distance, produced by ion exchange with propylamine, was used in order to achieve the intercalation of larger gentamicin molecule. ZrP-GTM intercalation product was prepared by using a 1:1 α-ZrP:gentamicin molar ratio.

A colloidal dispersion of exfoliated zirconium phosphate was prepared by titrating dropwise 5 g of α-$Zr(HPO_4)_2 \cdot H_2O$ suspended in 330 mL of water with 165 mL of 0.1 M propylamine solution at room temperature and under vigorous stirring for 1 h. Then, a solution of HCl was added to the colloidal dispersion until complete exchange of the propylammonium ions with protons, regenerating the acid form [31]. In a second step, 18.9 g of gentamicin sulfate was added to the gel and the mixture was stirred for 24 h at room temperature until complete exchange of the protons with gentamicin antibiotic. The reaction mixture was centrifuged, washed twice with deionized water and dried in oven at 40 °C.

2.4. Composite Production

The production of bioactive composites with PEOT/PBT and ZrP-GTM were carried out with a Lab Scale Corotating Twin Screw Extruder installed in the Nadir Laboratory, with a screw diameter of 11 mm and a length-to-diameter ratio (L/D) of 40. The screw profile is composed of 8 zones with three interposed kneading sections.

PEOT/PBT pellets were fed in the main hopper with a volumetric feeder. In addition, ZrP-GTM was fed in the main hopper using a double inlet.

The screw rotation speed was fixed at 80 rpm while the barrel temperature was set at 140 °C for the first zone and temperatures ranging from 145 to 150 °C for the following zones.

Nanocomposite wires were recovered at the die exit, solidified in air and after pelletized with a pelletizer machine. Using this approach, PEOT/PBT composites with 5, 10 and 20 wt % of ZrP-GTM were prepared. About 1 g of pellets of each prepared composite was melted at 80 °C and pressed in order to produce platelet disks for the x-ray diffraction and field-emission microscopy analyses.

2.5. Analytical Procedures and Instrumentation

The X-ray diffraction (XRD) patterns were recorded with a Philips X'PERT PRO MPD diffractometer (Amsterdam, Netherlands) operating at 40 kV and 40 mA, step size 0.0170 2θ degrees, and step scan 20 s, using the Cu Kα radiation and an X'Celerator detector (Philips, Amsterdam, Netherlands).

High performance liquid chromatography (HPLC) was carried out using the procedure reported in the article of Chuong et al. [32]. A precise amount of ZrP-GTM was weighted and then GTM was extracted from the solid. 0.2 g of ZrP-GTM was contacted with 10 mL of a solution of HCl/KCl 3 M under sonication for 30 min, in a graduated flask. Then, water was added until the exact volume of 50 mL. HLPC was performed on the obtained solution after filtration.

Thermogravimetric analyses (TGA) were carried out with an STD Q600 thermal analyzer TA Instrument (New Castle, United States), in air flow with a heating rate of 10 °C/min.

The morphology of the samples was investigated with an FEG LEO 1525 (Zeiss, Oberkochen Germany) scanning electron microscope (FE-SEM). FE-SEM micrographs were collected by depositing the samples on a stub holder and after a sputter coating with chromium for 20 s.

The Biocidal activity of PEOT/PBT neat polymer (as reference) and composites with 10 and 20 wt % of ZrP-GTM were evaluated with the AATCC145 Test Method on extruded filament.

3. Results and Discussion

3.1. ZrP-GTM Characterization

The ZrP-GTM intercalation compound was characterized by XRPD and TGA analyses. Figure 2 shows the XRPD pattern of ZrP and ZrP-GTM. The pristine ZrP in acid form has an interlayer distance

of 7.5 Å [33], while the ZrP-GTM presents an increased interlayer distance of 17.6 Å compatible with the intercalation of gentamicin.

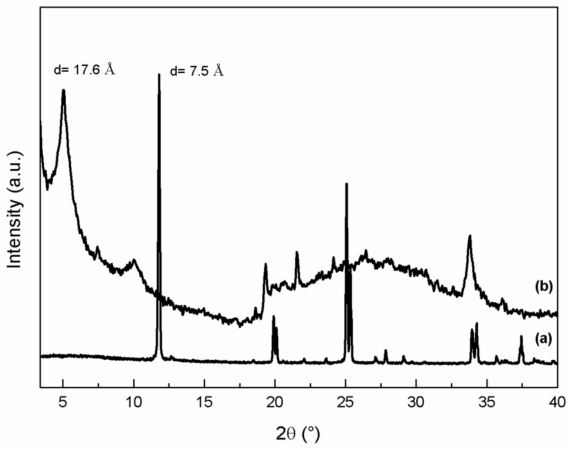

Figure 2. XRPD patterns of ZrP (**a**) and ZrP-GTM intercalation compound (**b**).

TGA curves of ZrP-GTM and pristine ZrP are reported in Figure 3. The overall weight loss of ZrP-GTM sample (b) was higher compared to the pristine ZrP material (a). ZrP shows two main weight losses: the loss of hydration water occurred in the range 20–200 °C, while the step between 200 and 800 °C is attributed to the loss of water due to the condensation of the HPO_4 groups. In addition, the thermogravimetric curve of ZrP-GTM sample shows two main steps in the ranges 20–200 °C and 200–800 °C: the first is due to the loss of water, while the second is attributed to the thermal decomposition of gentamicin and the condensation of the phosphate groups to produce zirconium pyrophosphate. From the difference in the weight loss corresponding to the second step of the TG curve, it was possible to calculate the gentamicin content. The experimental gentamicin content in the ZrP-GTM intercalation compound was about 54 wt % corresponding to the following chemical formula: $Zr(HPO_4)_{1.21}(PO_4)_{0.79}(GTM)_{0.79}\cdot 2H_2O$. The GMT loading in the solid obtained by HPLC analysis, carried out according to a suitable method reported in literature [32] was 53,% confirming the value calculated by TGA and excluding the presence of residual propylammonium.

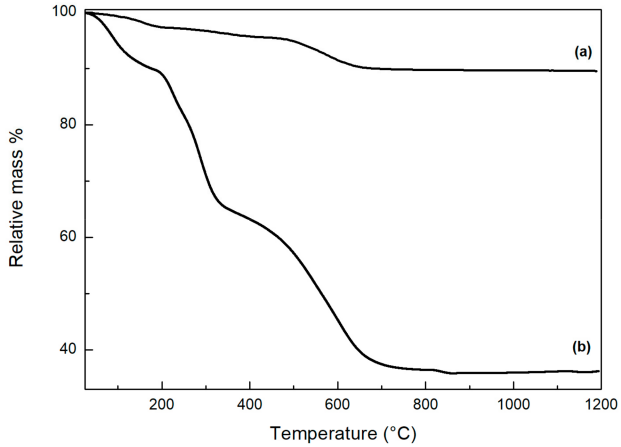

Figure 3. Thermal analysis (TGA) of α-ZrP (**a**) and ZrP-GTM intercalation compound (**b**).

Figure 4 shows SEM micrographs of the intercalation compound. The sample has a lamellar morphology with irregular shape and micrometric dimensions.

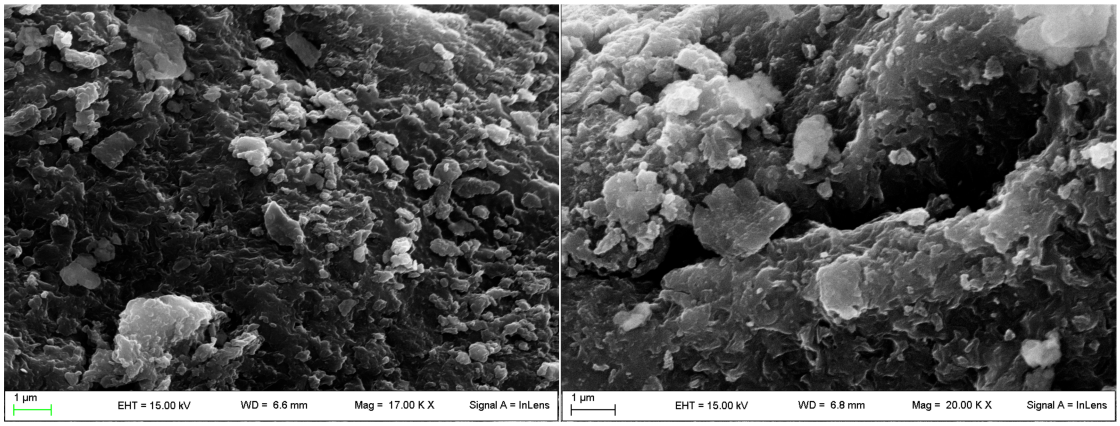

Figure 4. FE-SEM micrographs of ZrP-GTM.

3.2. Composite Characterization

The polymer composites were prepared, as described in the experimental section, by melt extruding PBT/PEOT with increasing amounts of the synthesized fillers. In detail, composites with 5, 10 and 20 wt % of ZrP-GTM were realized.

3.2.1. PBT/PEOT and ZrP-GTM Composites Characterization

Composites with 5, 10 and 20 wt % of ZrP-GTM were characterized by XRD analysis, to determine the dispersion degree of the filler (Figure 5). No signals of the lamellar filler were detected suggesting a good compatibility between the organo-modified filler and the polymer. The organic modification of the filler with gentamicin seems to produce a good dispersion and confers to the masterbatches antibiotic activity. In order to deeper understand the structure of the composites, they were analyzed by FE-SEM. The three composites presented the same morphological properties; for sake of simplicity in Figure 6, only the micrographs of the PBT/PEOT ZrP-GTM 20 wt % sample are reported. Despite the XRD analysis, it is possible to observe that, in the polymer matrix micrometric, aggregates attributable to the ZrP-GTM filler are still present; therefore, the obtained composites can be classified as micro-composite [34].

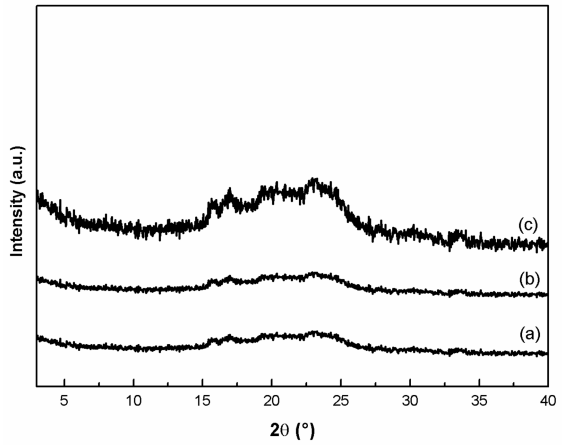

Figure 5. XRD of the composite samples: (**a**) PBT/PEOT ZrP-GTM 5 wt %; (**b**) PBT/PEOT ZrP-GTM 10 wt %; (**c**) PBT/PEOT ZrP-GTM 20 wt %.

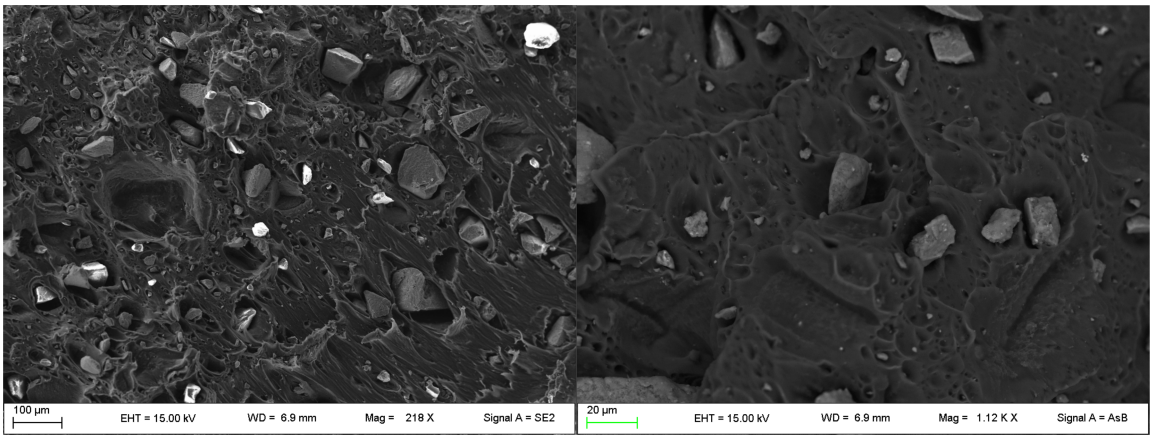

Figure 6. FE-SEM micrographs of the PEOT/PBT ZrP-GTM 20 wt % composite.

3.2.2. Antimicrobial Activity

The biocidal activity of the composites was evaluated. The analysis was carried out by monitoring the growth of *Staphylococcus epidermidis* (Gram+), identified as one of the bacteria responsible for septic scaffolding failure in polymer implants [35].

Pure PBT/PEOT and composites with 10 and 20 wt % of ZrP-GTM were evaluated using the AATCC145 Test Method, which is a qualitative test for detection of the antimicrobial activity [36]. The method was applied to a precise and weighed quantity of polymer wires, as obtained at the exit of die extruder. In Figure 7, the experimental results of the analysis are reported. *Staphylococcus epidermidis* colonies correspond to the yellow lines perpendicular to the polymer wires. From the photos, it is evident that, with the neat polymer, the growth of bacteria is diffused; therefore, no antimicrobial effect can be detected for the pure polymer (as expected). In both composites, with 10 and 20 wt % of ZrP-GTM, respectively, the growth of the bacteria is inhibited; in fact, near the wire areas, no colonies are detected confirming the biocidal activity of the composites. Since PEOT/PBT is a bioresorbable polymer [22,23], it is expected that the polymer will slowly dissolve/degrade in the biological medium freeing up the ZrP-GTM included in the composite material. The biocidal activity can be attributed to the ability of ZrP-GTM to slowly release the gentamicin [21], which possesses a well-known antibiotic activity [37].

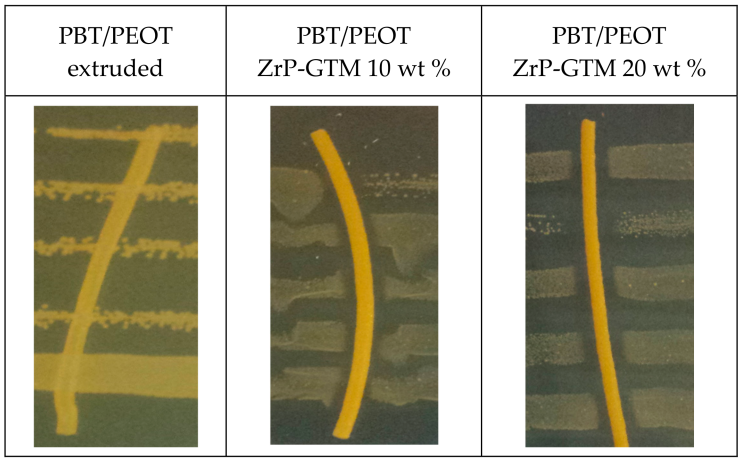

Figure 7. Photos of the indicated samples after the AATCC145 antimicrobial tests: (**left**) pure PBT/PEOT; (**center**) PBT/PEOT ZrP-GTM 10 wt % and (**right**) PBT/PEOT ZrP-GTM 20 wt %.

4. Conclusions

In this article, the preparation of antimicrobial composites based on a 3D printable and bio-resorbable polymer and an organic modified zirconium phosphate using melt compounding extrusion processing is reported. The use of melt compound approach for the composites preparation can open many industrial applications because of the easier scale-up if compared to the classic solvent casting procedures. Samples containing growing quantity of ZrP-GTM were prepared and characterized; the composites possess biocidal activity against *Staphylococcus epidermidis*, opening an interesting potential application in the field of 3D printed implantable scaffolds. Other specific analytical characterizations will be performed in the future, in order to deeply understand the potentialities of this innovative composite material.

Author Contributions: Michele Sisani, Maria Bastianini and Annarita Petracci have designed, synthesized and characterized ZrP-GTM. Marco Scatto has prepared the composites using the twin-screw extruder. Paolo Scopece and Alessandro Patelli have contributed to the composites characterizations.

Acknowledgments: This article was possible thanks to H2020 funding, in the project—FAST "Functionally Graded Additive Manufacturing Scaffolds by Hybrid Manufacturing" (NMP07, GA No. 685825).

Conflicts of Interest: The authors declare no conflict of interest.

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