Food Packaging and Shelf Life 16 (2018) 77–85

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Extruded hybrids based on poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) and reduced graphene oxide composite for active food packaging

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ARTICLE INFO

Chemical compounds studied in this article:
Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (no item found)
reduced graphene oxide (no item found)
zinc oxide (PubChem CID: 14806)

Keywords:
Graphene
Hybrid
Extrusion
Physical properties
Antibacterial properties
Migration

ABSTRACT

Hybrid materials, formed by the incorporation of a reduced graphene oxide-zinc oxide (rGO-ZnO) composite at 3, 6 and 9 mass% contents, into glycerol-plasticized poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), were prepared by melt extrusion. The rGO-ZnO composite was synthesized by the simultaneous reduction of zinc diacetate and GO, at 20:1 ratio, and characterized. Changes in the infrared spectra, X-ray diffractograms and Raman spectra, obtained for the hybrids in comparison to PHBV, evidenced the formation of physical interactions between matrix and filler. The opposite effects exerted by the addition of plasticizer and rGO-ZnO contributed to slight variations in dynamic glass transition temperature. Bactericidal activity was found against *Escherichia coli* as a result of direct contact between bacteria cells and the hybrids surface. Migration tests to food simulants, with the exception of acetic acid solution, revealed that the amount of ZnO that migrated were bellow the upper level recommended for Zn^{2+}.

1. Introduction

In response to demands from consumers and other stakeholders, the packaging industry continuously develops new products for food packaging. Environment concerns and food spoilage are among the main challenges to be addressed. On this context, the use of biodegradable materials and of active food packaging has attracted increasing attention.

Biopolymers have emerged as an alternative to synthetic polymers, which are associated with the accumulation of plastics waste in the environment. Among these materials, some polysaccharides and their derivatives, poly(lactic acid) (PLA) and polyhydroxyalcanoates (PHA) are the most investigated for packaging applications.

Polyhydroxyalcanoates (PHA) are a group of biocompatible and biodegradable aliphatic polyesters, synthesized from renewable resources by bacteria, such as *Ralstonia eutropha* and *Azobacter chroococcum*, in which they are deposited as insoluble inclusions in the cytoplasm. Poly(3-hydroxybutyrate) (PHB) and the copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) are produced commercially. In a recent report, PHBV was quoted as appropriate to be used as food contact material because of its structural and chemical stability (Chea, Angellier-Coussy, Peyron, Kemmer, & Gontard, 2016). To minimize costs and improve properties, the development of blends (Enriquez, Mohanty, & Misra, 2017) and composites (Malmir, Montero, Rico, Barral, & Bouza, 2017; Maiti, Batt, & Giannelis, 2007) was suggested by several authors.

Foodborne diseases, caused by microorganism contamination of food products, constitute a motive of concern. Active packaging consists of a new concept, devised to overcome this problem by extending or improving the shelf-life of packaged food. To achieve this aim, these systems usually incorporate components that release antimicrobial or antioxidant substances to the packaged food or, alternatively, absorb substances, which may lead to deterioration of the food product (Realini & Marcos, 2014; Van Long, Joly, & Dantigny, 2016). Micro- or nanosized solid particles with antimicrobial properties (for instance silver, gold, titanium dioxide, copper oxide, zinc oxide) have been added to biopolymers, mainly because of their stability under the severe conditions used for polymer processing (Sung et al., 2013). Several authors reported the effective antimicrobial activities of such biopolymer composites (Castro-Mayorga, Fabra, & Lagarón, 2016; Díez-Pascual & Díez-Vicente, 2014; Salarbashi et al., 2016). Also, carbon allotropes and derivatives (exfoliated graphite, carbon nanotubes,

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https://doi.org/10.1016/j.fpsl.2018.02.002
Received 18 September 2017; Received in revised form 28 December 2017; Accepted 9 February 2018

graphenes, and graphene oxide) were considered as reinforcing (Barletta, Puopolo, Tagliaferri, & Vesco, 2016; Demitri, De Benedictis, Madaghiele, Corcione, & Maffezzoli, 2016; El Achaby et al., 2016) and antimicrobial additives (Mazaheri, Akhavan, & Simchi, 2014; Ordikhani, Farani, Dehghani, Tamjid, & Simchi, 2015; Pal, Dubey, Gopinath, & Pal, 2017). Moreover, because of the enhanced activity and low citotoxicity (Ji, Sun, & Qu, 2016; Rojas-Andrade et al., 2017; Shi et al., 2016; Tegou et al., 2016; Zou, Zhang, Wang, & Luo, 2016) graphene derivatives/metal oxide composites alone have also been incorporated to biopolymers for antibacterial applications. However, migration of particles from those composites, incorporated to the packaging material, into the food matrix is a critical issue, very rarely addressed in research works.

Generally recognized as safe (GRAS) by the Food and Drug Administration (FDA, USA) (Jin, Sun, Su, Zhang, & Sue, 2009), zinc oxide (ZnO) was used to prepare graphene oxide composites. Solution-cast films of poly(lactic acid) and graphene oxide-ZnO composites were shown to display high UV light-shielding efficiency and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, and were recommended for active packaging applications (Huang et al., 2014). Other authors immobilized chitosan on graphene oxide (GO) by covalent binding and, then, ZnO particles were deposited *in situ* on GO layers. The product exhibited very low minimum inhibitory concentrations (MIC) against *E. coli* and *S. aureus* (Chowdhuri, Tripathy, Chandra, Roy, & Sahu, 2015).

In this work, hybrids of PHBV and a reduced graphene oxide-zinc oxide (rGO-ZnO) composite were prepared and characterized by physicochemical methods. Contrarily to the majority of reported data, in which antimicrobial films have been prepared by casting, melt-processing – a much more convenient method for industrial production – was chosen for the preparation of the hybrids. The antimicrobial properties of the PHBV/rGO-ZnO hybrids against *Escherichia coli* were evaluated by two different methodologies. Bearing in mind the potential application of the hybrids as food packaging, simulants were used in migration experiments to detect ZnO possible release.

2. Materials and methods

2.1. Materials

Natural graphite flakes and zinc diacetate dihydrate were purchased from Sigma-Aldrich (São Paulo, SP, Brazil). Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), with 8 mass% hydroxyvalerate units, was donated by PHB Industrial (Serrana, SP, Brazil). The molar masses of PHBV ($\overline{M}_w$ = 184,930 and $\overline{M}_n$ = 56,230) were determined by gel permeation chromatography in chloroform at 25 °C, in a Shimadzu equipment (Tokyo, Japan). All other analytical grade reagents and solvents were purchased from Vetec Química Fina (Rio de Janeiro, RJ, Brazil) and were used as received. In all cases, ultrapure water from a Millipore Direct-Q 3 UV system was used.

2.2. Preparation of graphite oxide

Graphite oxide (GO) was prepared by the methodology described by Marcano et al. (2010). Briefly, a 9:1 mixture of concentrated H_2SO_4/H_3PO_4 (400 mL) was added to a 1 L flask. The flask was adjusted to an ice-bath, and 3 g of graphite (1 mass eq.), and then 18 g of $KMnO_4$ were added slowly to the acid mixture under stirring. The reaction conditions were maintained for 1 h. Then, the reaction mixture was heated to 50 °C and stirred for 12 h. The product was poured into a 1 L becher filled with ice cubes/water, and homogenized with 3 mL of 30% H_2O_2. The oxidized product was filtered, and washed exhaustively with ultrapure water, 30% HCl aqueous solution (~1200 mL), and ethyl alcohol (1200 mL). Finally, the resulting yellow-brownish material was filtered, and dried in an oven at 60 °C for 48 h.

2.3. Preparation of reduced graphene oxide-zinc oxide composite (rGO-ZnO)

The rGO-ZnO composite was prepared according to Hwa & Subramani (2014), with modifications. Briefly, GO (3 g) was dispersed in 600 mL water under stirring at 7000 rpm in an Ultra Turrax IKA T25 (IKA® Works, Wilmington, NC, USA) for 2 h. The dispersion was poured into a two-neck flask of 1 L capacity, to which 150 g of zinc diacetate dihydrate were added. The reaction mixture was maintained under stirring for 4 h, and the pH was adjusted to pH 10 with 1 M NaOH. Then, 18.5 g of sodium borohydride were added under stirring, which was maintained for other 30 min. Finally, the resulting reaction mixture was heated at ~130 °C for 6 h. The product (rGO-ZnO) was recovered by filtration, exaustively washed with water, and dried in an oven at 60 °C for 48 h.

2.4. Preparation of PHBV/glycerol/rGO-ZnO mixtures

To prepare PHBV/glycerol/rGO-ZnO mixtures, the rGO-ZnO composite was firstly dispersed in glycerol in an Ultra Turrax T25 at 7000 rpm, for 2 h. To ensure a better dispersion, the amount of glycerol varied depending on the content of rGO/ZnO to be added to PHBV. Accordingly, to prepare the mixtures of PHBV with the incorporation of rGO-ZnO at 3, 6 and 9 mass%, glycerol was added at 13, 16 and 19 mass % (based on PHBV mass). For some experiments, the hybrid with the incorporation of the rGO-ZnO composite at 1 mass% content, dispersed in glycerol at 10 mass% (based on PHBV mass) was also studied. Separately, the dispersions were added to 200 g of PHBV, homogenized in a conventional mixer at 400 rpm for 30 min, and maintained in sealed polyethylene bags for 2 days at 4 °C, before processing.

2.5. Processing

Extrusion processing was carried out in a Coperion ZSK 18 (Werner & Pfleiderer, Stuttgart, Germany) co-rotating twin-screw extruder, with a L/D ratio of 40 and seven heating zones. The seven heating zones were maintained at 160 °C–165 °C, and the screw speed was set at 200 rpm. The extruded materials were pelletized, ground and compression-molded by heating at 165 °C under 68.9 MPa for 10 min, and cooling at the same pressure for 5 min in a cold press.

2.6. Characterization of materials

FTIR analyses were carried out using a Perkin Elmer spectrometer, Frontier model (Waltham, MA, USA) at ambient temperature, using KBr disks, in the 4000–400 cm^{-1} region, with accumulation of 20 scans and 4 cm^{-1} resolution.

XRD patterns were performed in an Ultima IV diffractometer (Rigaku Corporation, Osaka, Japan) operating at the CuKα wavelength of 1.5418 Å, at 40 kV and 20 mA. The scattered radiation was detected at ambient temperature in the 0.6° to 80° (2θ) angular region at 0.5° (2θ)/min in the reflection mode. For a better visualization of characteristic reflections, the diffractograms were smoothed using the software OriginPro 8.0 (Savitsky-Golay, polynome: 2, points of window: 10). The d001 value of the stacking order of graphite oxide was calculated by the Bragg's Law (Eq. (1)).

$$n\lambda = 2\ d\ \text{sen}\ \theta \quad (1)$$

where n is an integer, λ is the wavelength of the incident wave (1.5418 Å), d is the spacing between any two atomic planes in the crystal lattice, and θ is the angle of reflection. The average crystallite size of the ZnO samples was obtained with the Debye-Scherer equation (Eq. (2)).

$$D = K\lambda/\beta \cos\theta \quad (2)$$