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Research paper

Development, characterization and cytotoxicity of novel silane-modified clay minerals and nanocomposites intended for food packaging

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ABSTRACT

Layered silicates are largely used to develop polymer nanocomposites with improved physical properties compared to the original polymeric matrix. In this work, the silylation of sodium montmorillonite (Mt) was performed in distilled water using 3-aminopropyltriethoxysilane and vinyltrimethoxysilane, obtaining two different silane modified clay minerals named Clay3 and Clay4, respectively. Clay3 indicated a better profile with a notable increase in the interlayer space compared to Mt. For this reason, Clay3 was selected to scale up its production and to be incorporated in polypropylene (PP) matrix. The PP-Clay3 nanocomposite showed an enhancement in elasticity, a reduction of 15% in the oxygen transmission rate and important antimicrobial effects in comparison to pristine PP. Also, for the first time, cytotoxic effects in human cell lines exposed to Clay3 and Clay4 were evaluated. No cytotoxic damage was observed after Clay3 exposure but an important decrease in cell viability was produced by Clay4. The PP-Clay3 nanocomposite is an attractive alternative for the food packaging industry although further work is needed to get better results in the case of Clay4. Moreover, individual case by case evaluation of clay minerals under different conditions is required in order to obtain the most complete toxicity information.

1. Introduction

Nanocomposite materials based on modified clay minerals imbued in polymers as reinforcements have been intensively studied in the last years aiming to obtain final products with enhanced mechanical, thermal and barrier properties for the food industry, specifically for the food packaging area (Smolander and Chaudhry, 2010). Among the layered silicates most used in this research area, montmorillonite (Mt) which is a 2:1 layered smectite, commercially known as Cloisite®Na$^+$ (CNa$^+$), stands out by its high aspect ratio, capacity for swelling and ion exchange, softness, high solvent resistance, good mechanical strength, high capacity for delamination and low cost (Betega de Paiva et al., 2008; Jordá-Beneyto et al., 2014).

Mt has 1-nanometer-thick layers when incorporated to a polymer, stacked on each other and loosely bound by Van der Waals forces, which allow the penetration of water and/or other polar molecules. To compensate the negative charges generated by the isomorphic substitutions which occur in the tetrahedral and octahedral sheets of the Mt, exchangeable cations such as Na$^+$, Ca^{2+} and Li$^+$ are electrostatically fixed in the interlayer space of the clay minerals (Bertuoli et al., 2014; Paul and Robenson, 2008). However, a simple dispersion of Mt in a polymer matrix will not produce a nanocomposite with better properties compared to the bulk material. This is due to the poor interfacial interactions between Mt hydrophilic reaction sites and the highly hydrophobic polymer chains (Pisticelli et al., 2010). The solution for the aforementioned disadvantages can be achieved by introducing organic groups; providing more supports to the interaction between clay minerals and polymers (Silva et al., 2011).

The most useful chemical modification procedure to prepare a more organophilic Mt is a cation exchange (Paul and Robenson, 2008; Pavlidou and Papaspyrides, 2008; Ray and Okamoto, 2003). One of the most commonly performed modification through cation exchange is by quaternary ammonium salts (Betega de Paiva et al., 2008; Jordá-Beneyto et al., 2014); however, due to the quaternary ammonium salt degradation at high temperatures through Hoffman-degradation, the thermal instability of the organo-clay mineral becomes a strong limitation in the processing of clay mineral/polymer nanocomposites (Wang et al., 2012; Xie et al., 2001, 2002).

For these reasons, the grafting silylation reaction of hydrophobic groups into the layer surfaces has attracted great attention. It is usually performed through the reaction between silane coupling agents and the reactive silanol groups which are located at the broken edges of the clay mineral layers and at the structural defects located at the interlayer and external surface (Di Gianni et al., 2008; Herrera et al., 2005; Pisticelli et al., 2010). The irreversible grafting reaction is the solution to obtain chemically modified clay minerals in which the molecule of the silane modifiers is bonded to the layers of Mt (Wypych, 2004). Several results

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have been obtained after Mt silylation reaction through different modification methods (Di Gianni et al., 2008; Pisticelli et al., 2010; Silva et al., 2011).

With respect to the toxicity of organosilane-modified clay minerals used in packaging the information is very limited, even non-existent, demanding a strict evaluation according to the authorities in charge of the regulation about new materials intended as food contact materials (EFSA, 2011, 2016). At the moment there is no data on organosilane-modified clay minerals already approved for this application in Europe, although other kinds of nanocomposites are already being used in the U.S. market (Plackett and Siró, 2012). Therefore, the development of novel nanocomposites is of interest to continue the innovation in the food packaging industry.

The present work studies the modification of Mt with two different silanes. The modifiers used were 3-aminopropyltriethoxysilane (APTES), obtaining Clay3, and vinyltrimethoxysilane (VTMS), giving Clay4, both silanes approved for food contact applications (Commission regulation (EU) No 10/2011). X-ray diffraction (XRD), thermogravimetric analysis (TGA) and Fourier transform infrared (FTIR) spectroscopy were employed to characterize the modified clay minerals. Furthermore, the process of modification of the organosilane-modified clay mineral with the highest reaction yield was scaled-up in a pilot plant reactor with the aim of obtaining a sufficient amount of such modified clay mineral to be incorporated to polypropylene (PP) in 4% *w*/*w* in order to develop novel nanocomposite materials. The modification reaction yield of the scaled up organoclay mineral was evaluated through XRD and TGA to be compared to the results achieved in the laboratory. The PP nanocomposite materials were used to obtain injected trays and their mechanical and barrier properties were tested. The advantages with respect to the non-reinforced polymeric matrices were highlighted and a shelf-life study of red meat packaged with these new packaging systems was carried out. Moreover, a toxicological study with the modified clay minerals was also performed, considering a cytotoxicity biomarker in human target cell lines from intestinal and hepatic origin.

2. Materials and methods

2.1. Supplies and materials

Polypropylene pellets were purchased from SABIC (Murcia, Spain) (PPQR615 grade) for the preparation of the nanocomposites.

Purified sodium Mt was purchased from Southern Clay Products Inc. (Texas, USA), with moisture content between 4 and 9%. The cation exchange capacity (CEC) of Mt is 92.6 mequiv/100 g. Silanes were supplied by Sigma Aldrich (Madrid, Spain). APTES and VTMS with ≥98% of purity were used for the preparation of the organosilane-modified clay minerals, Clay3 and Clay4, respectively.

Culture medium, fetal bovine serum and cell culture reagents were obtained from Gibco (Fisher Scientific, Madrid, Spain). Chemicals for the cytotoxicity assay were provided by Sigma-Aldrich (Madrid, Spain) and VWR International Eurolab (Barcelona, Spain). Protein reagent assay was obtained from BioRad (Madrid, Spain).

2.2. Organomodified clay mineral preparation

The silylation of Mt was performed according to Silva et al. (2011) with modifications. Briefly, 10 g of dry Mt were dispersed in 500 ml of distilled water at 80 °C during at least 2 h with the help of an UltraTurrax (IKA, Spain) working between 9000 and 11,000 rpm. Then, 5.7 ml of APTES or VTMS for Clay3 and Clay4, respectively, was added in each case. The resulting dispersion was stirred for 30 min under nitrogen atmosphere. The dispersion was left overnight at room temperature and the reaction product was washed with water at 60 °C and recovered by centrifugation at 3500 rpm. Then, the silane modified clays were dried between 60 and 70 °C during 24 h. Finally, the dried clay minerals were crushed and maintained other 24 h in the oven under vacuum.

According to the better results obtained, Clay3 was selected to scale up its production and develop the final nanocomposite. The silylation reaction with APTES was scaled up in a pilot plant reactor, with a capacity of 20 l, in order to obtain large quantities of the organosilane-modified clay mineral required for tray manufacture.

2.3. Compounding and injected sample manufacture

Nanocomposites with PP as polymeric matrix have been prepared by extrusion at A. Schulman Inc. facilities (Castellon, Spain), following a conventional melt mixing process. PP was selected as it presents better chemical, mechanical and heat resistances compared with other matrices, an important issue to develop the final nanocomposite product (British Plastic Federation, http://www.bpf.co.uk). Processing has been carried out in a twin screw extruder (COPERION, L/D 40, Stuttgart, Germany) equipped with a controlled feeding system to control the polymer/clay mineral ratio and desired throughput. PP pellets are added into the main hopper and 4% wt of dried Clay3 has been fed at 20D.

The extrusion conditions were as follows: (i) temperature range: this varied from 160 to 170 °C in the different barrel zones; (ii) screw speed: 350 rpm; (iii) throughput: 20 kg h^{-1}; (iv) pressure: 20 bar.

Once the molten material strands are extracted from the extruder die, they are cooled in a water bath. The water bath temperature was set and controlled at 21 °C. Once cooled, the strand was cut into pellets using a strand cutter (21 m/min) to provide pellets of 1 mm length.

Pellets obtained from the compounding process were dried using a Mann Hummel dehumidifier (TF40 ECO HT, Zaragoza, Spain) at 50 °C for 2 h and were subsequently used to manufacture the trays by injection moulding in a Battenfeld injection-moulding machine, L/D 22 (HM B6 model, Kottingbrunn, Austria).

2.4. Characterization methods of clay minerals and nanocomposite films

Clay minerals were characterized using different techniques: FTIR spectra were obtained on a Tensor 27 spectrometer (Bruker, Massachusetts, USA). For this purpose, an ATR (Golden Gate Single Reflection Diamond ATR, Specac, United Kingdom) objective was used. For each sample 64 scans were recorded with a resolution of 4 cm^{-1}. WAXD (wide-angle X-ray diffraction) analysis were performed on clay mineral powders (clay mineral pellets were obtained by pressing), using a Bruker AXS D5005 diffractometer equipped (Massachusetts, USA) with a Cu hollow cathode (l = 0.154 nm) and an oscillation detector. The X-ray diffraction pattern was measured from 1.5 to 40° (2θ) at a scan rate of 0.02 °C/min. TGA analysis was performed on clay mineral powders using a TA Q5000IR thermobalance (TA Instruments, New Castle, USA). A total of 7.0 mg of sample was heated from 25 to 900 °C at a heating rate of 20 °C/min in nitrogen atmosphere.

The mechanical properties of the injected trays were assessed on a universal testing machine M350-20CT (Testometric Lancashire, United Kingdom) through compression tests following the ASTM D2659-95 standard.

Barrier properties were also evaluated through the oxygen transmission rate (OTR) tests. The OTR evaluation was performed at 23 °C and 50% relative humidity with a MOCON OXTRAN (Minneapolis, USA) following the ASTM F1307 standard (Minneapolis, USA).

2.5. Red meat shelf life study and microbiological study

After obtaining the injected trays, 100 g/tray of sliced flank steak were packaged by using a packaging machine (ULMA Packaging, Alfafar, Spain) at 19 °C and 70% O_2/21% CO_2 of modified protective atmosphere conditions and sealed with a polyethylene/PP film. Four replicates of each reference were used and every tray was stored at 4 °C during 14 days.

Shelf life study of the red meat was evaluated from microbiological analysis on total aerobic mesophilic bacteria and determined on Plate