NANOFILLERS FOR FOOD PACKAGING APPLICATIONS: PROPERTIES, TOXICOLOGICAL PROFILE AND CITOTOXICITY

E. Araque[1], N. Ortuño[1], M. Jorda[1], C. Fito[1], S. Maisanaba[2], S. Pichardo[2], A. Jos[2], S. Aucejo[1]
[1]Packaging, Transport, & Logistics Research Institute (ITENE), C/ Albert Einstein,1. Parque Tecnológico. Paterna, Spain
[2]Area of Toxicology, Faculty of Pharmacy, University of Seville, Profesor García González nº2, 41012 Seville. Spain

Abstract. Nanocomposites are polymers reinforced using organic or inorganic nanometer-sized phase (nanofiller) which improve mechanical, thermal, barrier and other functional properties to develop innovative packaging solutions. The main goal of this work is to provide a better understanding of the relationship between some of the more relevant physical-chemical properties of nanofillers, selected according to their broadest commercial interest for application in the packaging industry, with their safety concerns and potential human hazardous effects. Emission and exposure real time measurements were performed at workplace to characterize the nanoparticle release during the processing of polymeric matrices with the studied nanofillers for obtaining the nanocomposite**.** Nanoparticle with lower potential hazardous effects based on its physical-chemical characterization and emissions at workplace was used as nanofillers to develop reinforced polylactide (PLA) beverage bottles with improved properties for which potential toxic effects on consumers using cytotoxicityticy biomarkers were performed.

Introduction. The effects of nanomaterials on human health may be influenced by their physico-chemical properties, determining the propensity of a material to generate airborne dust during its handling. The health risk potential of nanoparticles will depend on their nature, release and dispersion, magnitude and period of exposure to airborne nanoparticles, and also on exposure control in the workplace. Therefore, characterization of nanomaterials is a key issue in the health risk assessment for understanding their potential adverse effects on workers, consumers and the environment.
Nanoparticles have been identified as promising materials for the reinforcement of different materials. These nanofillers are able to improve mechanical, thermal, barrier and other functional properties of packaging materials. This is of great importance especially in biopolymers, since they present insufficient properties for their application in food packaging applications. Additionally, there is another concern related with the security of the nanofillers on consumers, and a lot of research is being developed nowadays in this issue.

Materials and Methods
The nanofillers have been selected according their broadest applicability in the packaging industry. The selection include: a metal (Ag), a metal oxide (ZnO,) and an organoclay (ModClay, developed in ITENE).

1. *Physical-chemical characterization of nanoparticles*

Redox potential of suspensions of nanoparticles in H_2O was measured by difference of potential between two electrodes (platinum and Ag/AgCl reference electrode). All samples were at 0,1M, except ModClay, that were at 6000 ppm.

2. *Measuring and monitoring of airborne nanoparticles at workplace:*

Measurements started with the warm-up of the twin screw extruder and continued until nanocomposite compounding was completed. Such airborne nanoparticles parameters were measured using a Condensation Particle Counter (CPC- Model 3007, TSI) in the range from 10-1000 nm, and a Philips Aerasense NP monitor (Nanotracer).

3. *PLA masterbach preparation and bottle injection*

Different compositions of PLA masterbach were produced using PLA pellets blended with 4% of the ModClay. To produce large scale compound, a Coperion twin screw extruder DSE 20/40 equipped with a side feeder for powder dosing was used. Bottles were processed in the injection–blowing equipment. The process used to obtain the bottle consisted of one step injection-blowing procedure. Processing temperatures profile was set between 200 and 230 ºC.

4. *Characterization of reinforced bottles*

Barrier properties of the bottles were performed following standard ASTM E96. Seven bottles were filled with 100 grams of calcium chloride, previously dried at least 4 hours at 240ºC, and were closed using the torquemeter settled at 15 lbf.in. Measurement conditions were 23ºC and 75 % relative humidity.

5. *Cytotoxicity*

Cellular viability of the human intestinal cell line Caco-2 exposed for 24 and 48h, to the migration extract from a PLA + ModClay nanocomposite in cell culture medium was determined using MTS metabolization and Neutral Red Uptake as biomarkers. Migration extract was obtained through commission regulation EU No. 10/2011; “plastic materials and articles intended to come into contact with food”.

Results

Redox potential: ModClay, with higher redox potential, is the nanoparticle with less ability to transfer electricity between the electrodes, while Ag Nanoparticles have the biggest one (Table 1).

Table 1. Redox potential results in water.

Samples	E(V) vs Ag/AgCl in H_2O
Zinc Oxide Nanofillers (ZnO)	0.246
ModClay	0.195
Silver Nanoparticles (NPs)	0.404

Quantification of airborne nanoparticles: Higher values of inhalation exposure (Number of particles/cm^3) were obtained for Ag while behaviour of ZnO and ModClay were similar.

Water vapour transmission rate. Results are shown in Table 2. It can be observed the difference between the raw PLA bottles and the bottles with the additive, reaching in the best case an improvement of 15%.

Table 2. Water Vapour Transmission Rate Results for the PLA bottles.

Sample	WVTR (grH_2O/bottle·day)
PLA Bottle	0,070
PLA_4%ModClay	0,062

Cytoxicity assessment

Results obtained in the cytotoxicity study showed no toxic toxic effects in the Caco-2 cell line in any of the biomarkers assayed in the range of concentration tested (from 0 to 100% migration extract in cell culture medium).

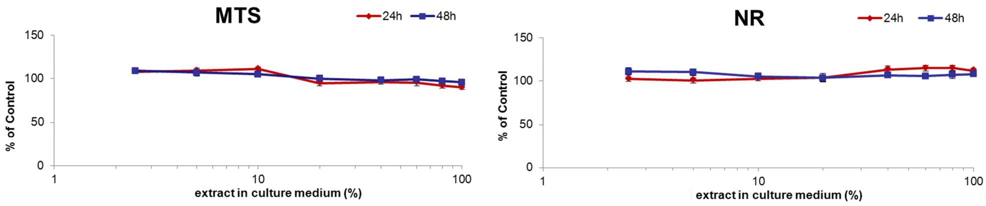

Figure 2. Results from cytotoxic assay of the migration extract.

Conclusions

Physico-chemical characterization and workplace measurements of three nanoparticles were studied for determining as a preliminary stage the potential risk of the studied nanoparticles. ModClay presented lower values of specific surface area and redox potential in water, suggesting lower hazard risk than Ag or ZnO. Moreover, particle concentration measurements at workplace showed that local exhaustive ventilation during the studied extrusion processing of nanocomposites contributes to generate a safer workplace in the case of ModClay processing. Therefore, ModClay was selected as nanofiller for developing reinforced PLA bottles. PLA bottles showed an improvement in properties; mechanical, thermal and barrier, showing a great potential for packaging applications.

Finally, cytotoxicity tests of migration extracts of ModClay bottles were tested on Caco-2cells. Results showed that studied reinforced PLA bottles presented absence or low cytoxicity. It can be concluded from the point the view of improved properties, safety at workplace, and toxicity in human cell, that ModClay is promising nanofillers for the reinforcement of food packaging materials.

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