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# Evaluation of antioxidant and antimicrobial properties of biocompatible low density polyethylene/polyaniline blends

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ABSTRACT

Melt processed blends of linear low density polyethylene (LLDPE) and polyaniline, in nanorod form, synthesized via the "falling pH" method (NR-PANI), with 5%, 10%, 15% and 20% NR-PANI loading, were evaluated for active packaging applications. The oxygen permeability decreased as the amount of NR-PANI in the blends increased. The blends films also exhibited antimicrobial and antioxidant capabilities. The oxidation of fish oil was delayed in the presence of the NR-PANI/LLDPE blends, and there was a negative correlation between oxidation and the NR-PANI content in the films. Moreover, the blends films were biocompatible to mammalian cells, meaning that NR-PANI/LLDPE films can potentially be utilized for a range of active packaging applications.



## 1. Introduction

Packaging is an integral part of the food sector. It has helped globalize the food industry by providing food preservation and protection systems so that long distance transfers of food stuffs can be facilitated (Lim, 2011). Socio-economic demands for hygienic, safer and cost effective packaging materials have brought about considerable developments in the area of packaging. One innovation that is attracting increased attention is active food packaging systems. Active food packaging systems consist of active agents such as antioxidants or antimicrobials, either contained in sachets, functionalized on the surface or incorporated directly in the packaging matrices (Bolumar et al., 2011; Camo et al., 2008; Kerry et al., 2006; Nerin, 2010; Restuccia et al., 2010). These systems have the capability to interact dynamically with the products or their immediate environment to enhance product shelf life, beyond simply providing an inert barrier to external elements (Hutton, 2003). While the presence of antioxidants helps lower the rate of rancidity leading to lipid oxidation and brown coloration, antimicrobial agents lessen food spoilage by pathogenic microorganisms (Kerry et al., 2006; Perez-Perez et al., 2006; Quintavalla and Vicini, 2002).

Various active packaging systems, containing synthetic as well as natural additives, have been reported in the recent literature. Flat extruded ethylene vinyl alcohol copolymer films containing green tea extract (Lopez de Dicastillo et al., 2011a; Lopez de Dicastillo et al., 2012) or beta-cylcodextrins (Lopez de Dicastillo et al., 2011b), which exhibit antioxidant properties, have been proposed for active packaging applications. Polylactic acid films containing α-tocopherol and butylated hydroxytoluene (BHT) (Byun et al., 2010), low density polyethylene (LDPE) films containing natural antioxidants derived from barley husk (Pereira de Abreu et al., 2011) and polyethylene terephthalate (PET) trays sprayed with citrus fruit extracts (Contini et al., 2011) have also shown antioxidant properties. Moreover, soy protein isolate coated polypropylene/polyethylene films (Gamage et al., 2009), thymol and carvacrol incorporated polypropylene films (Ramos et al., 2012), polyethylene and polyethylene polyamide composite films coated with sorbic acid (Hauser and Wunderlich, 2011) and polypropylene films activated with plasticized proteins (Lee et al., 2008) have exhibited antimicrobial properties. However, it would be useful to incorporate a single active material showing both antioxidant and antimicrobial properties in food packaging matrices.

Polyaniline, an intrinsically conducting polymer, is gaining recognition for its antimicrobial (Gizdavic-Nikolaidis et al., 2011; Shi et al., 2006) and free radical scavenging properties (Hsu et al., 2008; Hsu et al., 2011; Nand et al., 2011a; Nand et al., 2011b). The oxidation of food stuffs is induced by oxygen-containing radicals. Therefore, scavenging of the radicals, upon formation, can be an effective strategy to preclude the oxidation of food stuffs as the propagation of the oxidation reaction is prevented (Nerin et al., 2008). Thus, incorporating polyaniline in polymer matrixes such as LDPE, one of the most widely used packaging materials in the

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food sector (Ornelas-Paz et al., 2012), has the potential to yield antioxidant as well as antimicrobial active packaging systems.

Polyaniline is a low cost, environmentally stable and easy to prepare conducting polymer (Golczak et al., 2008). It can exist in a continuum of oxidation states and be easily switched between the reduced and oxidized states. Polyaniline is oxidized in the presence of free radicals and in doing so neutralizes the free radicals (Nand et al., 2011a). Owing to its lower oxidation level, polyaniline prepared via the "falling pH" method (NR-PANI) exhibited superior free radical scavenging capacity compared to regular polyaniline formed in the presence of a strong acid (Nand et al., 2011a). NR-PANI also has the thermal resilience necessary for the thermal processing of polymers on a large scale, and has exhibited high free radical scavenging capacity after being subjected to temperatures up to 200 °C (Nand et al., 2011b; Nand et al., 2012a). Recently antioxidant NR-PANI/PET blends were processed at an even higher temperature of 265 °C (Nand et al., 2012b). The assessment of polyaniline biocompatibility is of continued interest for the application of conducting polymer in medical devices, but is also applicable to food packaging applications in considering toxicity issues. A recent examination of polyaniline powders showed positive results with regards to a lack of skin irritation, but some cytotoxicity was evident with certain cell lines, which was linked to small molecular by-products in the polyaniline synthesis that could be removed through additional purification steps (Humpolicek et al., 2012).

The objective of the current study was to evaluate the antioxidant and antimicrobial capabilities of melt processed NR-PANI/linear LDPE (LLDPE) blends for potential active packaging applications. The spectroscopic characterization, thermal and mechanical properties, microscopy and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity of the blends have been reported in an earlier communication (Nand et al., 2012c). The results of biocompatibility, antimicrobial, oxygen radical absorbance capacity (ORAC), NR-PANI leaching, oxygen permeability and accelerated aging of fish oil tests on the NR-PANI/LLDPE blends are presented here.

## 2. Experimental

### 2.1. Materials

Aniline, obtained from Sigma–Aldrich, was distilled under reduced pressure of 240 mm of Hg and stored under a nitrogen environment at 5 °C before use. Methanol, ammonium persulfate, fluorescein sodium salt, sodium thiosulphate, potassium iodide, chloroform, acetic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), phosphate buffered saline (PBS), phosphate buffer tablets, resazurin sodium salt and Tween 80, also purchased from Sigma–Aldrich, were used as received. For mammalian cell culture experiments using murine fibroblast cell line L929 (ATCC CCL-1), Dulbecco's Modified Eagle Medium (DMEM), 0.4% Trypan blue solution and fetal calf serum (FCS) were obtained from Life Technologies. Cell culture was performed in 24-well tissue culture plates (TCP) (Nunc) and fluorescence readings were taken in black 96-well plates (Perkin Elmer). 25% Glutaraldehyde was obtained from Scharlau. For bacterial culture of *Staphylococcus aureus* ATCC 6838, Difco tryptic soy broth (TSB) and Difco trypticase soy agar (TSA) were purchased from Fort Richard (Auckland). 2,2′-azobis-2-methylpropionamidine dihydrochloride (AAPH) was purchased from Merck. Ropufa fish oil was supplied by Invita Ltd (New Zealand). LLDPE resin (FC21HN) having a melt flow index of 1.0 g (10 min)$^{-1}$ and 0.918 g cm$^{-3}$ density was obtained from TCL Hunt Ltd (New Zealand).

### 2.2. Polyaniline synthesis

NR-PANI, consisting of micro/nanorods and flake-like structures, was synthesized by the "falling pH" method whereby 250 mL of 0.3 M ammonium persulfate solution was added to 250 mL of Milli-Q water containing 4.6565 g of distilled aniline with mechanical stirring at 5 °C for 24 h (Nand et al., 2011a). The pH of the reaction mixture, during the synthesis, dropped from an initial 6.5–3.0 after 3 h, and to 1.3 over the 24 h reaction period. The reaction mixture was then filtered, washed several times with water and methanol, and the residue was dried at 40 °C overnight in a convection oven. The dried NR-PANI was ground to a fine powder and sieved using a sieve with 106 μm mesh size.

A more regular polyaniline sample, G-PANI, having a granular morphology (Nand et al., 2011a) was synthesized as NR-PANI but in the presence of hydrochloric acid (HCl). The ammonium persulfate solution was added to 100 mL of 1 M HCl containing 4.6565 g of aniline. The polymerization reaction and treatment of the product was performed as described for NR-PANI.

### 2.3. Film preparation

Blending of LLDPE and NR-PANI, with 5%, 10%, 15% and 20% loading of NR-PANI, designated as PEPA5, PEPA10, PEPA15 and PEPA20, respectively, was undertaken using a Brabender DSE25 twin screw extruder with an L/D ratio of 42. LLDPE pellets were dried in a Moreto dehumidification dryer at 80 °C overnight and NR-PANI was dried overnight at 60 °C in a vacuum oven prior to blending. LLDPE and NR-PANI were manually mixed and quickly fed into the extruder operating at 150 °C with a screw speed of 20 rpm at a torque of 200 Nm. The molten blends were extruded through a 3 mm die as a rod, cooled in air and pelletized. For comparison purposes, a control sample of LLDPE without any NR-PANI was also extruded as described above. The extruded pellets were compression molded between Teflon sheets at 150 °C to obtain 0.35 ± 0.05 mm thick films. The pellets were heated in the press for 1 min with repeated application and release of pressure to remove air bubbles, and kept at 10 kPa for an additional 4 min. The films were cooled in a flow of air immediately after removal from the hot press and stored in air tight containers until further characterization.

### 2.4. Leaching studies

4 × 4 cm$^2$ pieces of the film samples were immersed in 20 mL of Milli-Q water with occasional stirring. 1 mL aliquots were removed after 2, 7, 14 and 21 days and replaced with 1 mL fresh Milli-Q water. UV spectra of the leachates were obtained using a Shimadzu UV-1700 UV–vis spectrophotometer. 10 mg NR-PANI and G-PANI were dispersed in 10 mL water for 2 days and the spectra of the filtrates were obtained for comparison.

### 2.5. Oxygen transmission rate (OTR) measurement

50 cm$^2$ film samples were tested using a Mocon Ox-Tran 2/10 oxygen permeability system. The OTR was determined at 23 ± 0.2 °C and 2.5% relative humidity with 100% oxygen as the permeate. The samples were conditioned in the instrument for 3 h before commencing the measurements. All samples were analyzed at least in duplicate.

The oxygen permeability values of the samples were calculated using Eq. (1).

$$P = \frac{OTR}{\Delta p} \times l \quad (1)$$