

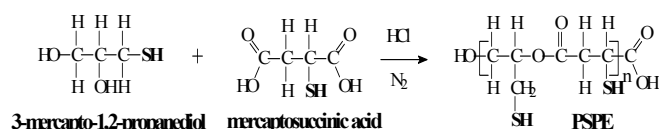
## Synthesis, characterization and antibacterial activity of nitric oxide-releasing poly(nitrosated) polyester/poly(methylmetacrylate) films

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**Statement of Purpose:** The emergence of multidrug resistant bacteria is broadly studied and appointed as a world health problem. Nitric oxide (NO), which is synthesized in biological systems, plays a key role in host defense through its antimicrobial actions. Herein, it is reported the synthesis and characterization of a novel biomaterial comprised of poly(nitrosated)polyester/poly(methylmetacrylate) (PNPE/PMMA), in which the SNO groups are covalently attached to the polymer chain. The NO release from PNPE/PMMA films was found to have antibacterial activity against one the major agents of hospital-acquired infections: *Pseudomonas aeruginosa* (*P. aeruginosa*) strains.

**Methods:** Polysulfhydrylated polyester synthesis (PSPE): PSPE was prepared by the polycondensation reaction between 3-mercapto-1,2-propanediol with a dicarboxylic acid mercaptosuccinic acid in the presence of HCl, as represented in Scheme 1.



**Scheme 1.** Polyesterification reaction yielding PSPE.

**Preparation of PSPE/poly(methylmetacrylate) (PMMA) (PSPE/PMMA) films:** Briefly, 70 mg of PMMA were dissolved in 1.0 mL of acetonitrile, followed by addition of 50 mg of PSPE. The solution was transferred to moulds and solid films of PSPE/PMMA (42:58 wt/wt) were obtained by casting, after solvent evaporation.

**S-nitrosation of PSPE/PMMA films:** PSPE/PMMA films were immersed in acidified sodium nitrite solution. The formation of S-nitrosothiol (-SNO) groups in the PNPE/PMMA was monitored at  $\lambda = 336$  nm, which corresponds to the absorption band of the S-NO bond.

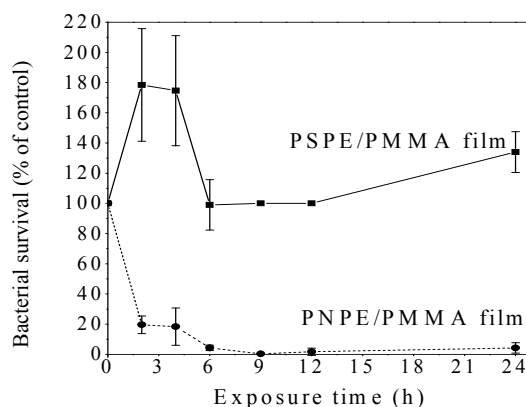
**NO release from PNPE/PMMA film:** The NO release from solid PNPE/PMMA films immersed in aqueous solution was monitored as nitrite formation by using the Griess reaction, as previously described (Seabra *et al.*, 2005).

**Antibacterial activity:** The antibacterial activity of the NO-donor PNPE/PMMA films was evaluated against *P. aeruginosa* strains, which are resistant to the most used antibiotics. Briefly, single colonies were dissolved in saline solution (NaCl 0.85%, wt/v) and adjusted to 0.5 index in McFarland scale ( $10^8$  colony formation units per mL (cfu.mL<sup>-1</sup>)). Then, the bacteria were diluted in Mueller Hinton broth and plated at density of  $10^5$  cfu.mL<sup>-1</sup> in 24-well plates. Three groups of analyzes were determined: growth control; PSPE/PMMA and PNPE/PMMA films. The plates were incubated at 37 °C for 24 h. The content of each well was serially diluted in

saline solution and plated in Mueller Hinton agar media. All plates were grown during 24 h at 37 °C and total cfu were counted.

**Results:** *NO-release from PNPE/PMMA films:* The kinetic of NO released from solid films of PNPE/PMMA films immersed in aqueous solution (Griess solution) showed an initial burst of NO release in the first hour, which corresponds to an initial rate of NO release of 33 mmol g<sup>-1</sup> h<sup>-1</sup>. The rate of NO release, after the first hour, and up to 24 h, was reduced to 0.68 mmol g<sup>-1</sup> h<sup>-1</sup>. These results show that films of PNPE/PMMA immersed in aqueous solution spontaneous release free NO for more than 24 h at a nearly constant rate, after the initial burst in the first hour.

**Antibacterial effect of PNPE/PMMA against *P. aeruginosa*:** Figure 1 shows the kinetics of bacterial growth curve during 24 h of incubation of *P. aeruginosa* exposed to PNPE/PMMA film and PSPE/PMMA film, as the control. PNPE/PMMA films had an effective time-dependent antibacterial effect with a reduction of cell viability > 98%. This effect can be attributed to their ability to release NO, which was shown to exert a powerful antibacterial effect.



**Fig. 1.** Bacterial growth curve during 24 h of incubation of *P. aeruginosa* exposed to PNPE/PMMA film.

**Conclusions:** The new NO-donor biomaterial synthesized in this work was able to control the growth of *P. aeruginosa* strain, which is a multidrug-resistant bacteria (resistant to the antibiotics currently used in treatments, such as Amicacyn, Gentamicyn, Imipenem, Cyprophloxacin and Cefotaxym). These results prove the potential of this biomaterial as an alternative approach in the antibacterial therapy.

**References:** Seabra AB, da Silva R, de Oliveira MG. Biomacromolecules, 6:2512-2520 (2005).

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