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TOXIC EFFECT OF TWO POLYMERS ON GRAM-NEGATIVE BACTERIA STRAINS

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Introduction

The increase of the population and their socio-economic development is linked to the industrialization development. The widespread utilization of surfactants such as personal care, detergents, textile, pharmaceutical and electronic could be toxic for the environment as well as for the living organisms because their increased amount and their chemical structure diversity. In spite of surfactants accumulation into the environment, the organisms developed mechanisms to fight the effect of pollutants by spontaneous mutations and activation of various defense mechanisms. Moreover, organisms react differently to pollutants according to their species, but their defense mechanisms could be very similar from prokaryotes to eukaryotes.

Herein, we decided to focus our attention on conjugated self-assembled systems in water which are promising materials for green-solvant-processable organic electronics. As such, oligothiophenes-based surfactants have been the subject of intense research due to their environmental stability, unique redox electrical behavior and ease of synthesis. They also generally exhibit low toxicity. To investigate in more details this latter issue, we evaluate the toxic effect of two cationic thiophene-based conjugated polyelectrolytes on the environment using a bacterial biological model.

Materials and methods

Chemical compounds: Two polymers were synthesized by ICGM team, as follows: 1) a phosphonium-based conjugated homopolyelectrolyte (P3HTPMe$_3$) and 2) a block copolymer containg a P3HT neutral block and ammonium-based P3HT block (P3HT-*b*-P3HTNMe$_3$). Initially, the solubility tests of homopolymer and the block copolymer were performed in water and chloroform.

Growth medium: Tryptone soya agar (Oxoid, UK) and Lauryl Sulphate Broth (Himedia Laboratories Pvt.Ltd) were purchased from AMS 2000 Trading Impex (Bucharest, Romania).

Bacteria strains: Three gram-negative bacteria *Citrobacter freundii* (ATCC 8090), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) were purchased from ATCC.

Bacterial assay: Bacteria was initially grown on tryptone soya agar then a single colony from each bacterial strain was incubated O/N in 5 ml Lauryl Sulphate Broth at

37°C and 130 rpm (New Brunswick Scientific, Innova 44). 0.4 OD600nm (measured by a spectrophotometer VWR International, USA) from each bacterial strain was incubated 4 h at 37°C in absence (control) or presence of various concentration of P3HTPMe$_3$ and P3HT-*b*-P3HTNMe$_3$, respectively (Clariostar Microplate Reader, BMG Labtech).

Results and conclusions

The toxicity of two synthesized chemical compounds was tested on gram-negative bacteria strains in various concentrations (0 to 200 mg/l for the first compound and 0 to 100 mg/l for the second compound). The chloroform, used to solubilize the second compound, did not influence the bacteria growth during the inhibition experiment with P3HT-*b*-P3HTNMe$_3$.

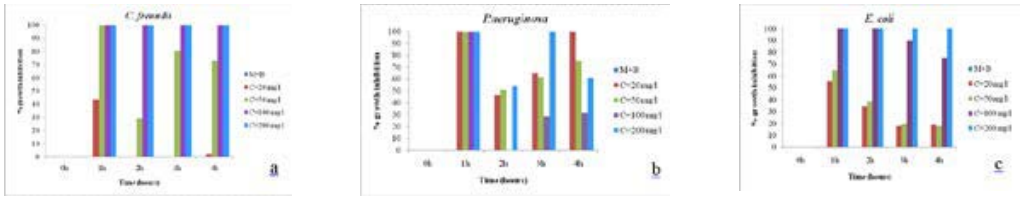

Figure 1. Toxicity effect of P3HTPMe$_3$ on gram-negative bacteria: a) *C. freundii*; b) *P. aeruginosa*; c) *E.coli; M+B=control*

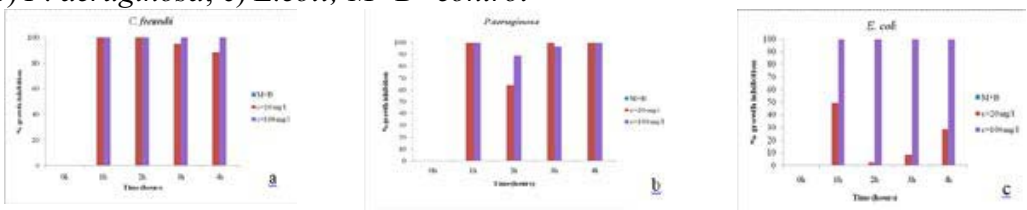

Figure 2. Toxicity effect of P3HT-*b*-P3HTNMe$_3$ on gram-negative bacteria: a) *C. freundii*; b) *P. aeruginosa*; c) *E.coli; M+B=control*

The gram-negative bacteria strains showed different resistance patterns when exposed to P3HTPMe$_3$ or to P3HT-*b*-P3HTNMe$_3$. 100% growth inhibition was observed at 100 mg/l and 200 mg/l of P3HTPMe$_3$ for both *C. freundii* and *E.coli*, respectively (Figure 1 a, c). *P. aeruginosa* developed particular defense mechanisms after 1h contact of P3HTPMe$_3$ when the growth inhibition decreased by half after 2h (Figure 1b).

P3HT-*b*-P3HTNMe$_3$ induced a high toxicity effect at 100 mg/l to all bacteria strains (Figure 2). Moreover, the growth of *C. freundii* and *P.aeruginosa* was inhibited at lower concentration of 20 mg/l (Figure 2a, b). Thus, the bacteria strains used, due to sensitivity of *C. freundii* as well as adaptation capacity of *P. aeruginosa* and *E.coli* makes them suitable biological models for further experiments.