Synthesis of polymeric ionenes and their antimicrobial and toxicological evaluation in vitro and in vivo

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Introduction

Polyionenes are ion-containing polymers with quaternary nitrogen atoms integrated into the polymer chain, providing inherent cationic charge density [1]. This feature allows them to interact with negatively charged microbial membranes, disrupting membrane integrity and exhibiting antimicrobial effects. To evaluate their antimicrobial properties, among the traditional MTT assay and microbiological methods, the Galleria mellonella (wax moth larvae) model is increasingly used due to its cost-effectiveness, ease of handling, and immune system similarities to mammals, without the need for ethical approval.

This study reports the synthesis, characterization, and antimicrobial evaluation of new polyionic polymers. Two synthesis methods were used: one involved the hydrolysis of poly(2-ethyloxazoline) and subsequent quaternization with bromoethane, and the second utilized the Menshutkin reaction with N,N,N',N'-tetramethylethylenediamine and 1,2-dibromoethane or bis(2-chloroethyl)amine hydrochloride.

Experimental

The synthesis of polyethyleneimine (PEI) involved hydrolysis of poly(2-ethyloxazoline) in hydrochloric acid at 100 °C, followed by precipitation using sodium hydroxide and purification through reprecipitation and freeze-drying. Quaternization of PEI was performed by reacting it with bromoethane in ethanol under reflux, followed by dialysis and freeze-drying. Ionenes were synthesized via the Menshutkin reaction by reacting TEMED with either 1,2-dibromoethane or BCAHC in N,N-dimethylformamide under reflux, with the resulting products purified by reprecipitation in diethyl ether and drying in a vacuum oven (Figure 1-3)

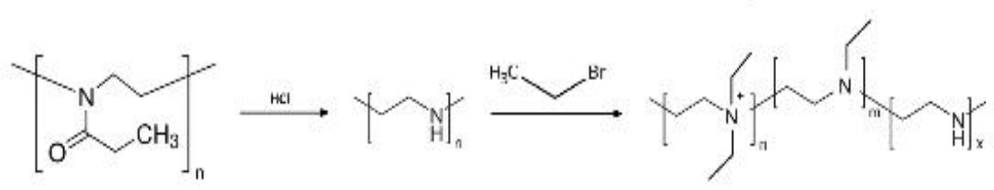

Figure 1. Synthesis of ionene-1 based on poly(2-ethyloxazoline) (DP1)

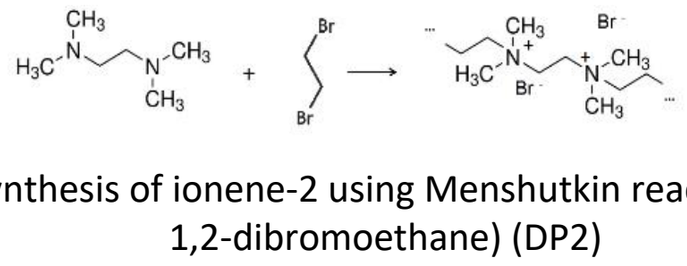

Figure 2. Synthesis of ionene-2 using Menshutkin reaction (TEMED and 1,2-dibromoethane) (DP2)

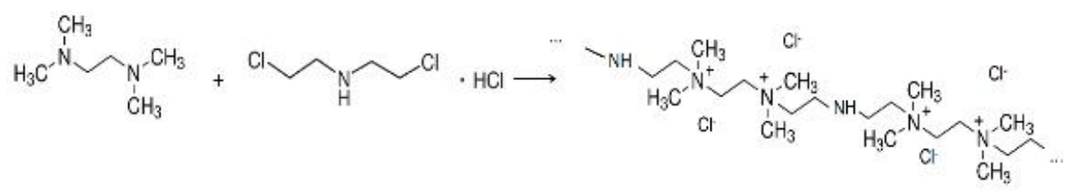

Figure 3. Synthesis of ionene-3 using Menshutkin reaction (TEMED and BCAHC) (DP3)

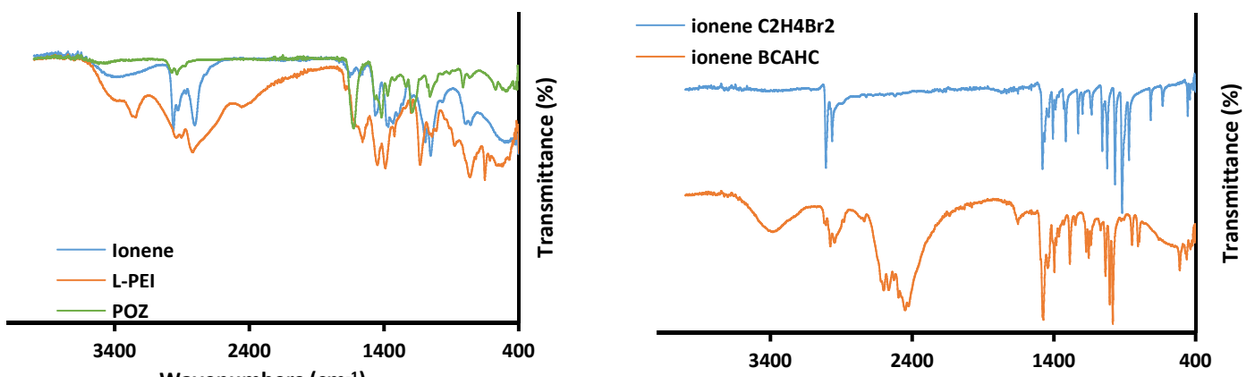

Figure 4. IR spectra for POZ, linear PEA, and ionene-1 (a) and Menchutkin-type ionenes-2 and 3 (b)

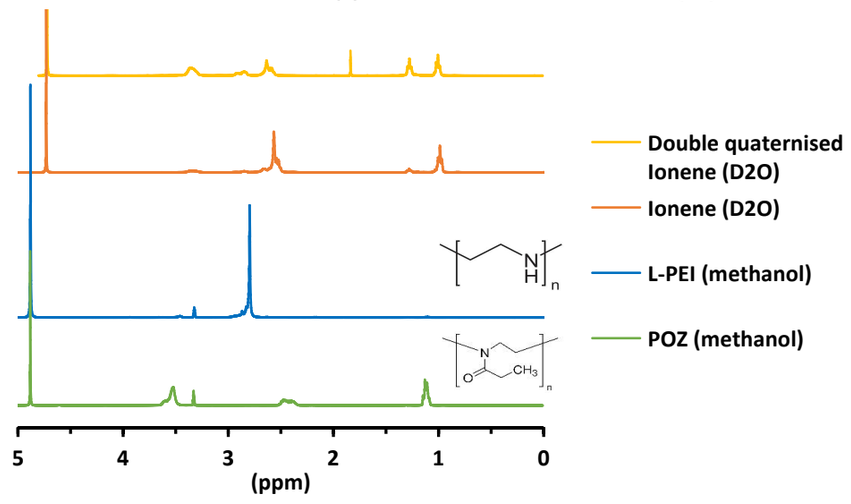

Figure 5. NMR spectra for POZ, linear PEA, and ionene-1

Results and discussion

Antimicrobial and toxicological evaluations were performed with the MTT assay, microbiological methods (minimum inhibitory concentration, and on Galleria mellonella larvae.

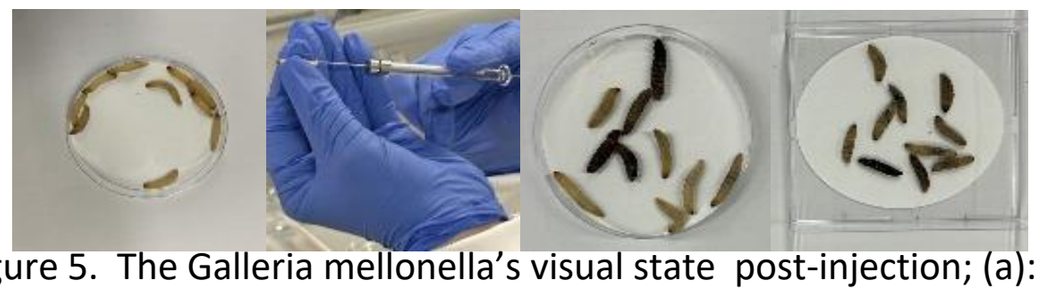

Figure 5. The Galleria mellonella's visual state post-injection; (a): alive and (b): dead

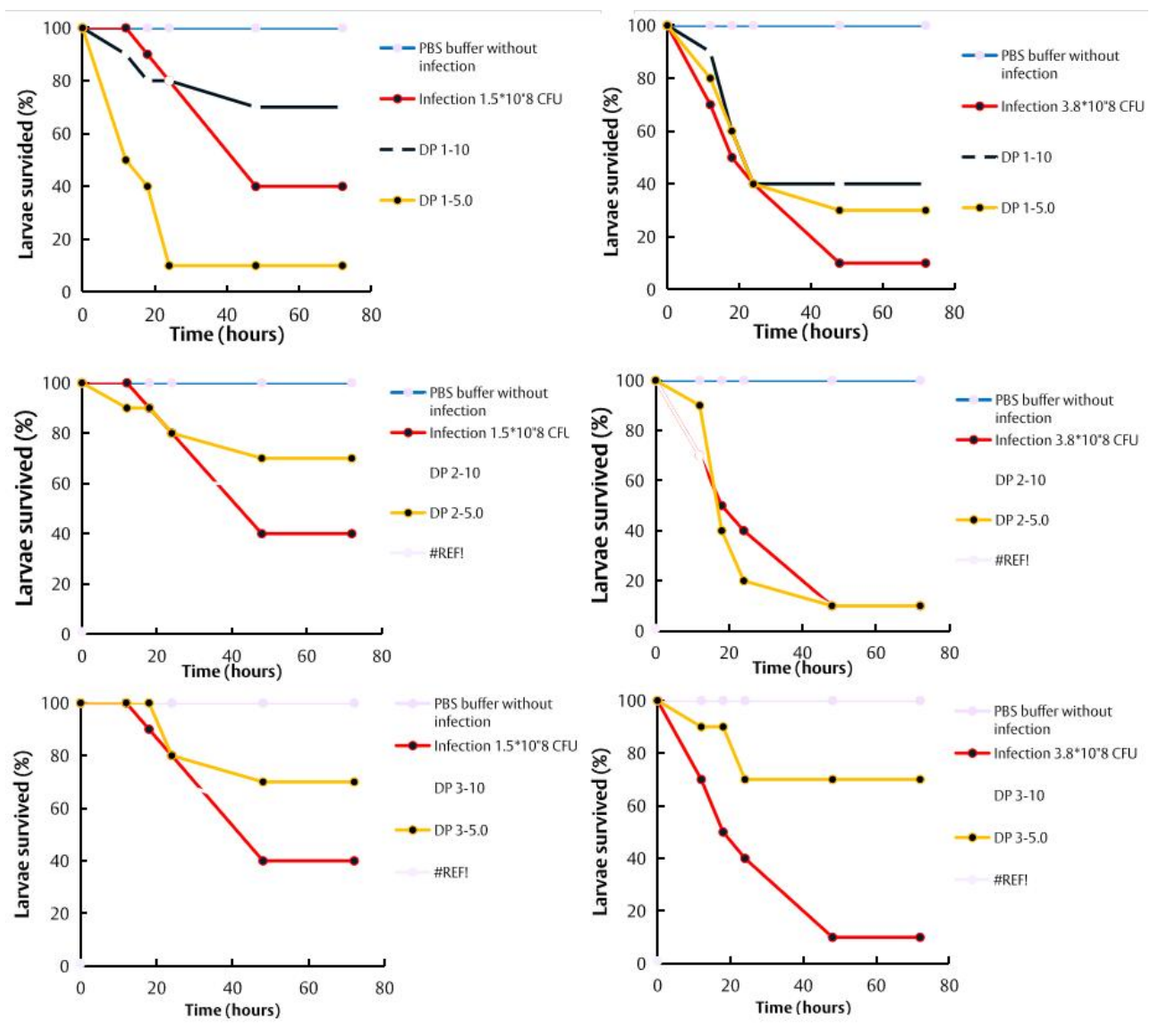

Figure 6. Survival of Galleria mellonella (wax moth larvae) subjected to an experimental infection with the *Staphylococcus aureus.*

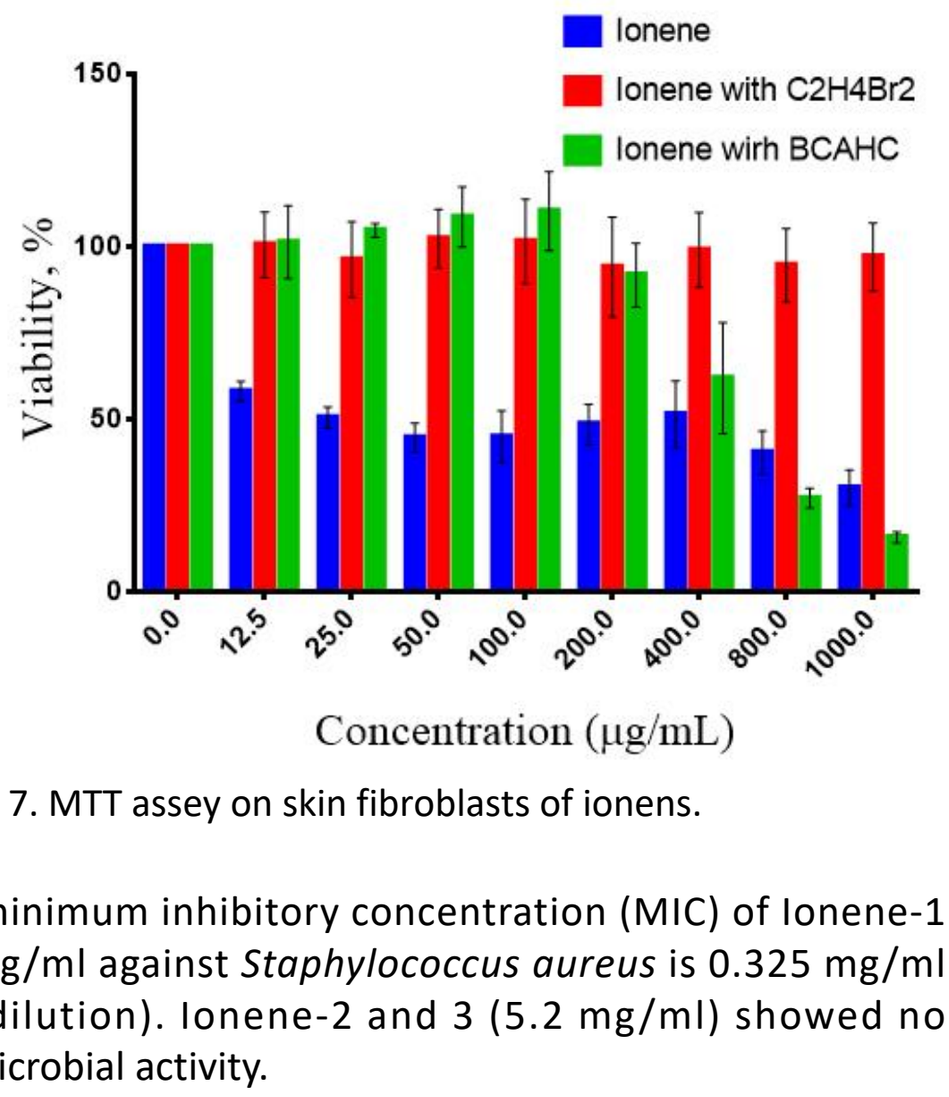

Figure 7. MTT assey on skin fibroblasts of ionens.

The minimum inhibitory concentration (MIC) of Ionene-1 5.2 mg/ml against *Staphylococcus aureus* is 0.325 mg/ml (1:8 dilution). Ionene-2 and 3 (5.2 mg/ml) showed no antimicrobial activity.

Conclusion

Three types of ionens were synthesised based on POZ and by Menshutkin reaction. Antimicrobial and toxicological evaluations were performed with the MTT assay, microbiological method and Galleria mellonella larvae. The MTT experiments demonstrated toxisity for ionene 1.The highest atimicrobial efficacy was observed with the ionenes 1 and 3 samples, suggesting their potential for further development.

References

1. S.R. Williams, T.E. Long Recent advances in the synthesis and structure-property relationships of ammonium ionenes. Progress in Polymer Science 2009, 34, 762. DOI: 10.1016/j.progpolymsci.2009.04.004