

Improving aqueous solubility and antibacterial activity of triclosan using re-dispersible emulsion powder stabilized with gold nanoparticles

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EXPERIMENTAL SECTION

SI 1. Minimum Inhibitory Concentration (MIC) and Minimum Biocidal Concentration (MBC) Assays

E. coli BAA-1161 and MRSA bacteria were adjusted OD_{600 nm} to have a value at 0.5. Thereafter the serial dilution of bacteria was performed to obtain the bacteria at ~1.40-1.80 CFU mL⁻¹. Next, TCS/PEG-B (no-dilution, 5x, 10x, and 20 dilutions), TCS/PEG-B/GNPs (no-dilution, 5x, 10x, and 20 dilutions), or TCS (0.17 mg) dissolved in PBS (1 mL) at a volume of 100 µL were added in a 96 well-plate. The prepared bacterial suspension (100 µL) was added to each well containing the test formulations. The MIC and MBC tests were conducted using modified protocols from previous publications.^{1, 2} A solution of resazurin dye (0.3 mg mL⁻¹) was

prepared and sterilized by passing it through a 0.2 µm membrane filter. Resazurin dye (30 µL) was added to each well and incubated for 24 h at 37 °C. The colour transformation from blue to pink was monitored. The MIC was determined based on the presence of blue resazurin. To determine the MBC at 0 and 24 h, 100 µL of bacterial sample from each well was spread on NA and incubated for 24 h at 37 °C. The MBC value was determined when no colony growth was observed from the directly plated contents of the wells.

RESULTS AND DISCUSSION

At 0 h, the blue resazurin colour remained unchanged in *E. coli* BAA-1161 and MRSA bacteria treated with no-dilution, 5x, 10x, and 20 dilutions of TCS/PEG-B/GNPs and TCS/PEG-B, and TCS because this MIC test required incubation time. Nevertheless, only non-diluted TCS/PEG-B/GNPs completely destroyed (MBC) *E. coli* BAA-1161. In the case of MRSA, all concentrations of TCS/PEG-B/GNPs completely killed MRSA excepting for 20x dilution of TCS/PEG-B/GNPs. Non-diluted and 5x dilution TCS/PEG-B completely killed MRSA at 0 h (Figure SIa). These results confirmed that TCS/PEG-B/GNPs had the highest efficiency in destroying *E. coli* BAA-1161 and MRSA bacteria.

Upon treatment with the test formulations for 24 h, *E. coli* BAA-1161 and MRSA exhibited no change in the blue resazurin colour. However, the control bacteria (*E. coli* BAA-1161 and MRSA without treating with the test formulations) appeared pink. All concentrations of TCS/PEG-B/GNPs completely killed *E. coli* BAA-1161 bacteria. In contrast, only the non-diluted TCS/PEG-B and its 5x dilution completely killed *E. coli* BAA-1161. Similar to *E. coli* BAA-1161, all concentrations of TCS/PEG-B/GNPs completely killed the MSRA after 24 h treatment. MSRA bacteria were completely killed after treating with all concentrations of TCS/PEG-B excepting for 20x dilution (Figure SIb). TCS dissolved in PBS had a small effect on inhibiting the growth of *E. coli* BAA-1161 and MRSA (Figure SIa&b).

Overall, the MIC/MBC tests provided the same direction of the results as the plate count agar approach, which was similar to a previous study.² These tests also confirmed that TCS/PEG-B/GNPs were highly effective in *E. coli* BAA-1161 and MRSA destruction due to bioviability of TCS in the formulation.

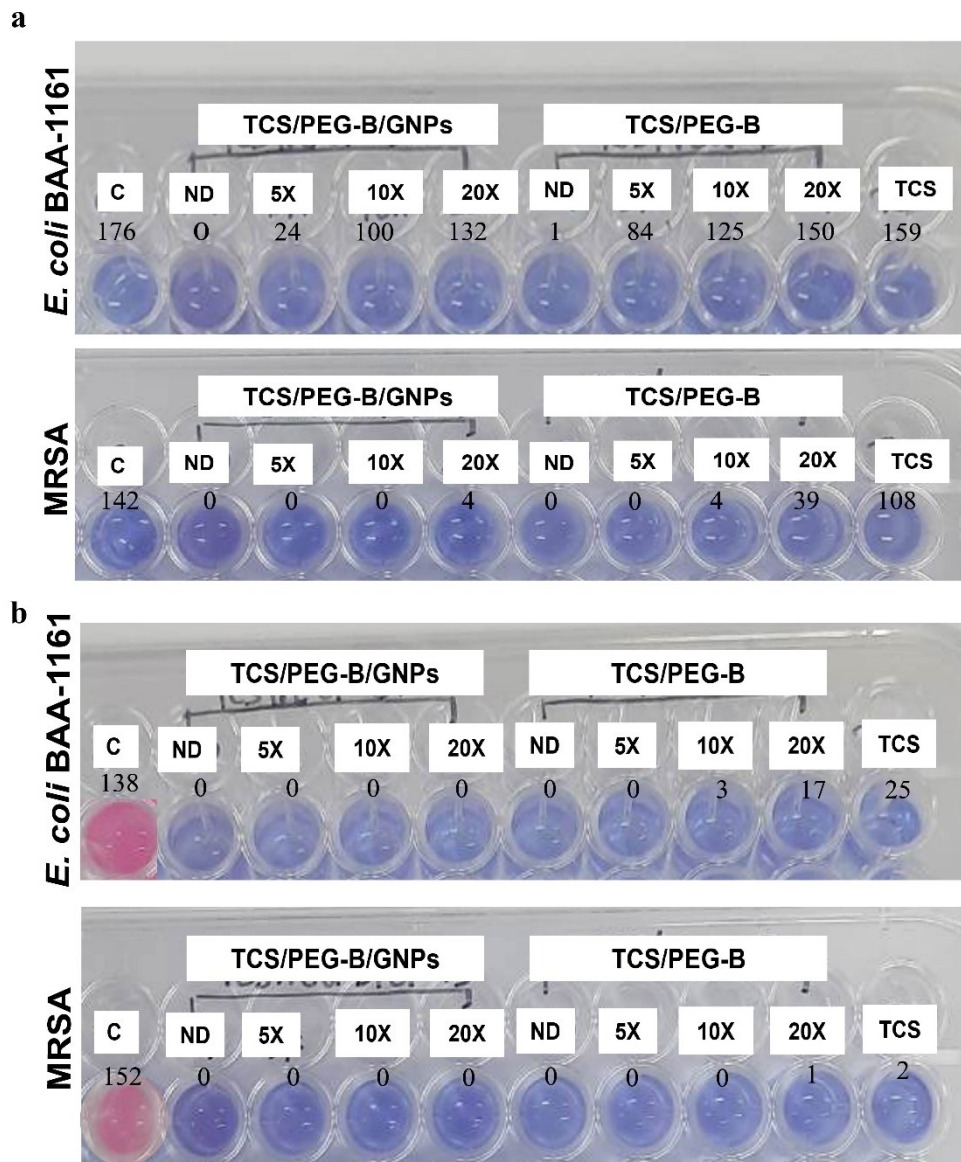


Figure S1. The MIC and MBC assays of *E. coli* BAA-1161 and MRSA treated with test formulations for (a) 0 h and (b) 24 h. The number at each well indicates the number of bacterial colonies present on the NA.

References

- (1) Elshikh, M.; Ahmed, S.; Funston, S.; Dunlop, P.; McGaw, M.; Marchant, R.; Banat, I. M. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. *Biotechnol. Lett.* **2016**, *38*, 1015-1019.
- (2) Costa, P.; Gomes, A. T. P. C.; Braz, M.; Pereira, C.; Almeida, A., Application of the resazurin cell Viability assay to monitor *Escherichia coli* and *Salmonella typhimurium* inactivation mediated by phages. *Antibiotics* **2021**, *10*, 974.