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Bactericidal effect of cinnamon leaf oil loaded onto chitosan microcapsules-modified biodegradable hydrogel-like films: an alternative for treating *Pseudomonas aeruginosa* infections

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Introduction

The multidrug-resistant *Pseudomonas aeruginosa* is considered a public threat, with antibiotics increasing its resistance. Essential oils (EOs) have demonstrated significant effects against several microorganisms. However, due to their volatile nature they cannot be delivered to the bacteria infected site in their free-state. Therefore, biodegradable polymeric delivery platforms are being engineered. Here, hydrogel-like films were produced from a combination of sodium alginate (SA) and gelatin (GN) to serve as delivery platforms for the controlled release of cinnamon leaf oil (CLO) entrapped within chitosan microcapsules. Chitosan microcapsules were prepared via ionotropic gelation with tripolyphosphate, containing at the core the CLO at MIC. Microcapsules were then embedded within a biodegradable SA/GN polymeric matrix processed via a solvent casting/phase inversion methodology with SA/GN used at 70/30 polymer ratio and 2 wt% SA concentration in distilled water. The coagulation bath was composed of a 2 wt% $CaCl_2$ aqueous solution. Qualitative and quantitative antimicrobial examinations validated the modified film potential to fight infections caused by *P. aeruginosa* bacteria.

Goal

Engineer a hydrogel-like film delivery platform that guarantees the controlled release of cinnamon leaf oil for the eradication of *P. aeruginosa*-derived infections.

CLO Encapsulation: Chitosan Microcapsules

Materials: Pure cinnamon leaf oil (CLO), nutrient broth (NB), nutrient agar (NA), gelatin (GN), sodium alginate (SA), Calcium chloride solution ($CaCl_2$), Chitosan (CS) 0,25 mg/mL solution, Tripolyphosphate (TPP) 0.25 mg/mL solution, Nile Red (NR) 0.1 mg/mL solution dissolved in ethanol.

CS1: 0.25 mg/mL CS + 0.25 mg/mL TPP (tripolyphosphate) + 39.3 mg/mL CLO + dH_2O+ acetic acid (AA)
CS4: CS1 with adjusted pH to 5.145

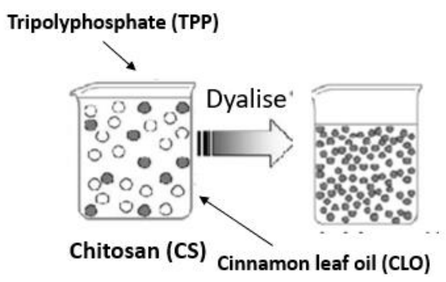


Figure 1. Schematic representation of the production of CS1/CS4 solutions.

Films Production

SA 2 wt% + GN 1 wt% + dH_2O + $CaCl_2$ 2 $wt\%$ +**CS1**
SA 2 wt% + GN 1 wt% + dH_2O + $CaCl_2$ 2 $wt\%$ + **CS4**
SA 2 wt% + GN 1 wt% + dH_2O + $CaCl_2$ 2 wt% - **used as a control**

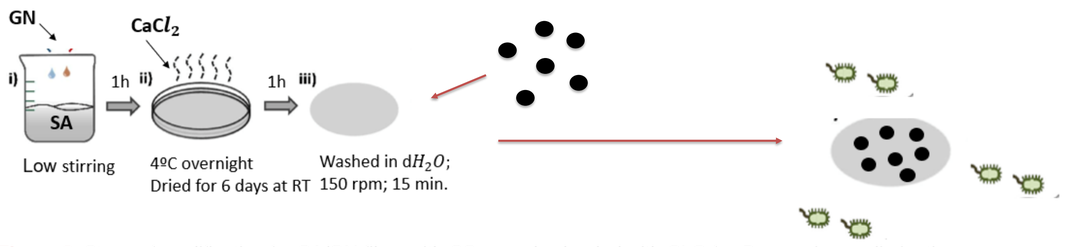
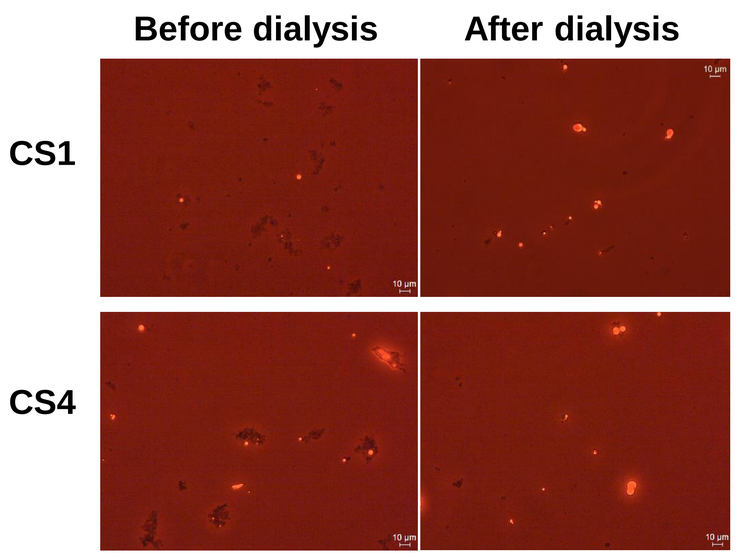


Figure 2. Steps of modification for SA/GN films with CS capsules loaded with CLO for *P. aeruginosa* elimination.

Microcapsules Morphology



Technique:
Fluorescence microscopy; 40x magnification (20 µm scale bar)
Observations:
- Elimination of the non-reacted polymer with dialysis.
- Greater microcapsule and unloaded chitosan aggregation on CS4 than on CS1.
- Microcapsules produced with no pH adjustment were more successful.

Figure 3. Microscopical observation of CS1 and CS4 capsules before and after dialysis.

Chemical Characterization

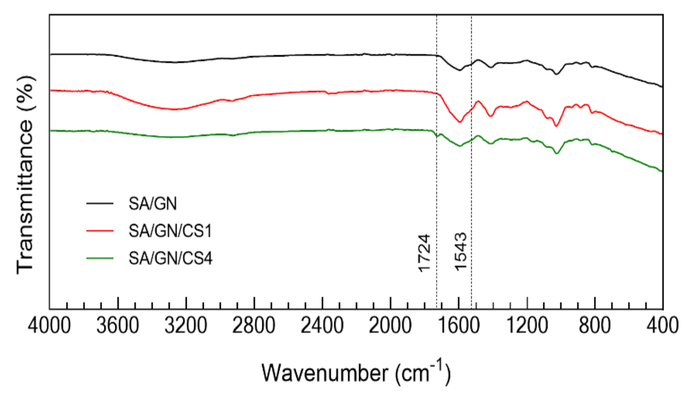


Figure 4. Transmittance as a function of the wavenumber for SA/GN; SA/GN/CS1 and SA/GN/CS4 samples.

CLO was more easily noticeable on the CS4-containing films than on the CS1, which may indicate that the EOs molecules were larger (formation of agglomerates).

CLO Release Profile

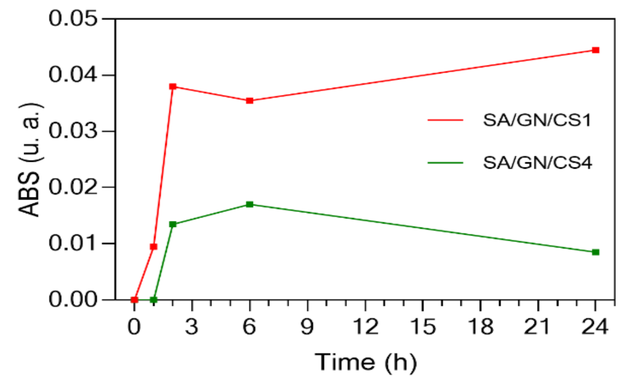


Figure 5. Absorvance as a function of time for SA/GN/CS1 and SA/GN/CS4 samples.

Lower release profile for the CS4-containing samples.

Antimicrobial Action

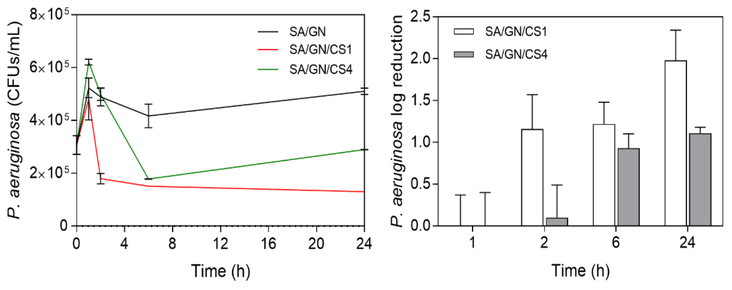


Figure 6. Time-kill kinetics and log reduction of the *P. aeruginosa* bacteria in contact with the unloaded and loaded films for SA/GN/CS1 and SA/GN/CS4 samples.

Time-kill kinetics and log reduction of the *P. aeruginosa* bacteria in contact with the unloaded and loaded films demonstrated the enhanced performance of the SA/GN/CS1 films in fighting bacteria in comparison with the SA/GN/CS4.

Conclusions

The incorporation of the CLO-containing CS microcapsules within the SA/GN fibers was confirmed;
The continuous release of the entrapped oil over a period of 24 h was attained, with a matched time kill kinetics against the *P. aeruginosa* bacteria;
CS1 loaded films were determined more effective than the CS4-loaded or the unloaded surfaces;
Future work will be aimed at improving the loading capacity and homogeneity of the microcapsules.

Acknowledgments
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