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Irradication of *Pseudomonas aeruginosa* via sodium alginate/gelatin films impregnated with chitosan microcapsules loaded with cinnamon leaf oil

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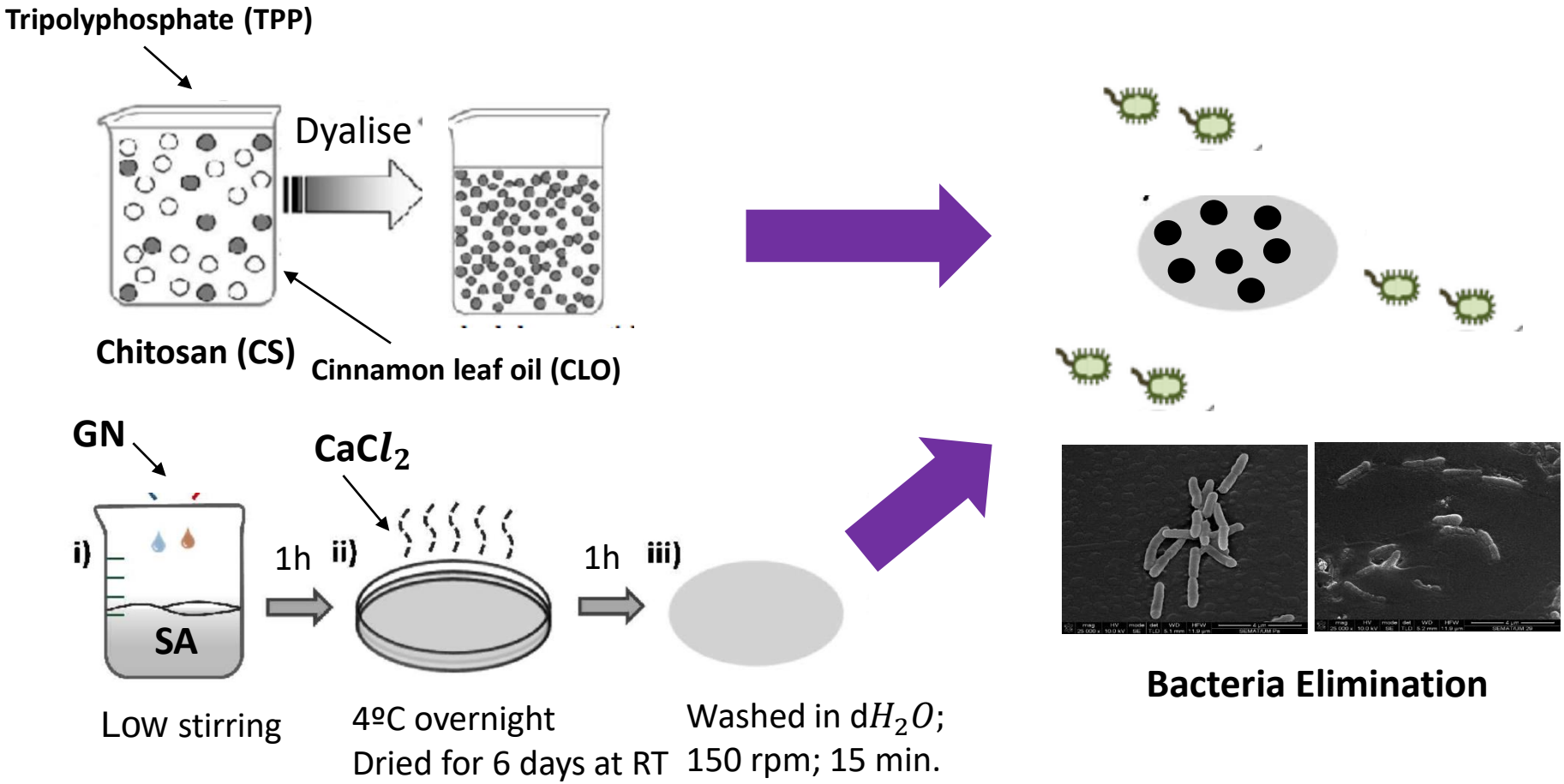
Irradication of *Pseudomonas aeruginosa* via sodium alginate/gelatin films impregnated with chitosan microcapsules loaded with cinnamon leaf oil

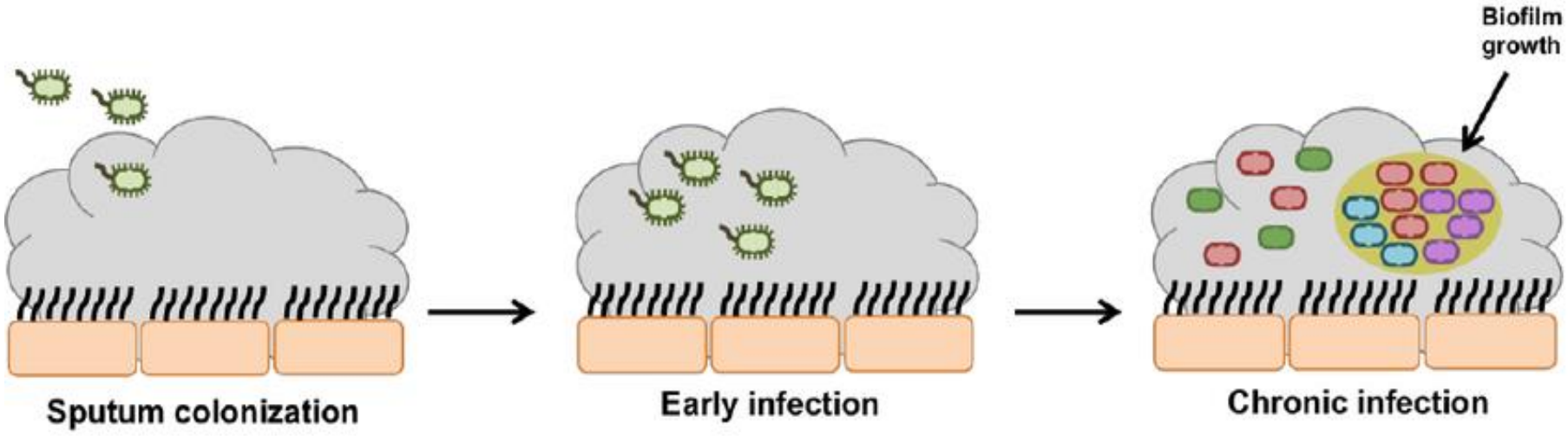


Abstract: The multidrug-resistant *Pseudomonas aeruginosa* is considered a public threat, with antibiotics increasing its resistance. Essential oils (EOs) have demonstrated significant effects against several microorganisms. However, due to their volatile nature they cannot be used in their free-state. 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. The minimum inhibitory concentration (MIC) of CLO was established at 39.3 mg/mL against *P. aeruginosa*. Chitosan microcapsules were prepared via ionotropic gelation with tripolyphosphate, encapsulating CLO at MIC. Successful production was confirmed by fluorescent microscopy using Nile red as detection agent. Microcapsules were 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 at 2wt% SA concentration. 2wt% CaCl2 was used as coagulation bath. The CLO-containing chitosan microcapsules homogeneous distribution was guaranteed by successive vortex and blending processes applied prior to casting. CLO controlled release from the films was monitored in physiological pH for 24h. Flexible, highly hydrated films were obtained, with the presence of loaded chitosan capsules being confirmed by FTIR. Qualitative/quantitative antimicrobial examinations validated the loaded film potential to fight *P. aeruginosa*.

Keywords: bio-based polymers; drug delivery platform; natural extracts; trigger-based release; bactericidal effects

Pseudomonas aeruginosa

Development of an infection by *P. aeruginosa*



Defined by the Infectious Diseases Society of America as a group of human pathogens with clinically relevant antibiotic resistance.

Buhl, M. et al. Expert Review of Anti-Infective Therapy (2015). https://doi.org/10.1586/14787210.2015.1064310

Treatments for bacterial infections

Antibiotics

Target bacterial functions and growth processes; bactericidal activity

Antibiotic resistant pathogens

Essential Oils

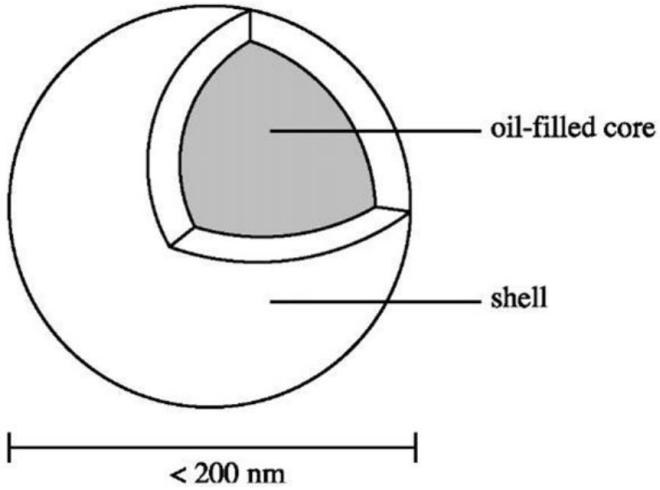
Mixtures of aromatic, volatile, lipophilic biomolecules, extracted from different regions of plants; strong anti-inflammatory, antiseptic, analgesic, spasmolytic, anesthetic and antioxidative properties

Citotoxicity at high concentrations; low resistance to degradation; high volatility

Felgueiras, H. P. et al. Biomolecules (2020). https://doi.org/10.3390/biom10081129

Nanocapsules

- Increased drug-loading efficiency
- Isolate the encapsulated payload from the surrounding
- Avoid degradation and burst release

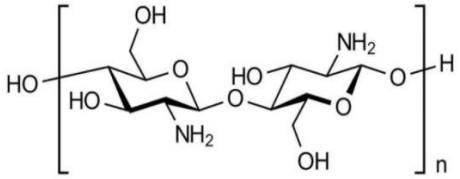


Structure of a nanocapsule

Deng S. et al. Nanomaterials (2020). doi: 10.3390/nano10050847

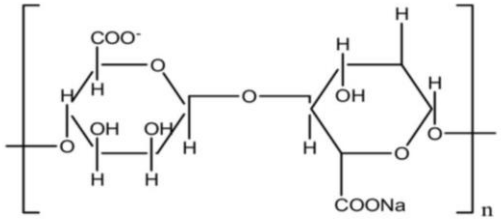
Polymeric shell

Chitosan



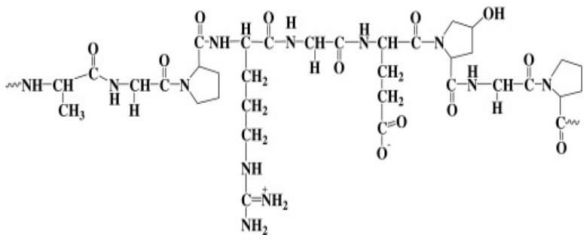
Biocompatibility;
Endogenous metabolizable degradable productions;
Gelation conditions;
Mucoadhesive properties;
The strong cationic surface charge of chitosan can lead to nanoparticle aggregation, protein adsorption.

Alginate



Fast ionic gelation with divalent cations;
Water-soluble salt;
Slow degradability;
Uncontrolled degradation kinetics.

Gelatin



Biodegradable protein;
Biocompatibility;
Water soluble.

Younes I. et al. Marine Drugs (2015). doi: 10.3390/md13031133
Sarker B. et al. Journal of Materials Chemistry B (2014). doi: 10.1039/c3tb21509a
Bigi A. et al. Biomaterials (2004). doi: 10.1016/j.biomaterials.2004.01.033

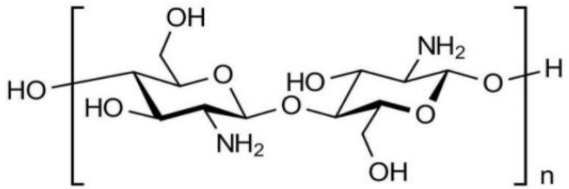
Goal

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

Microcapsules Production

Biodegradable Polymer:

Chitosan (CS)

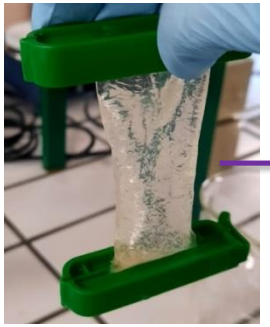


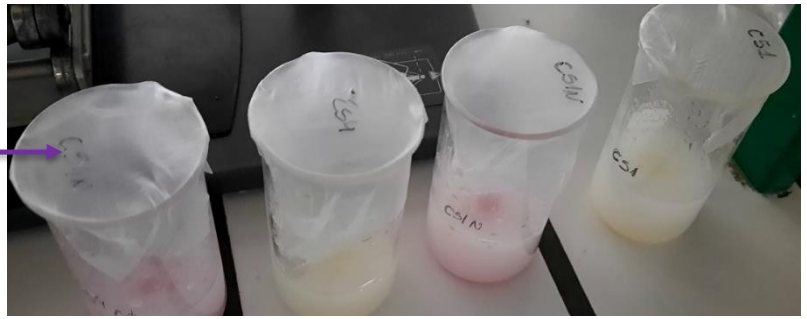
Polymeric solutions preparation:

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

Dialysis:

In a snakeskin dialysis membrane with 10,000 Da cut-off, for 24 h and 3 times bath exchange

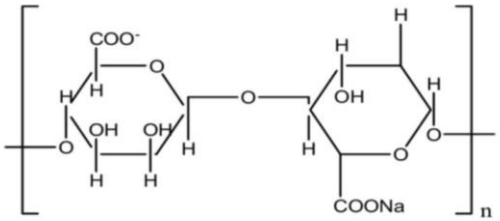




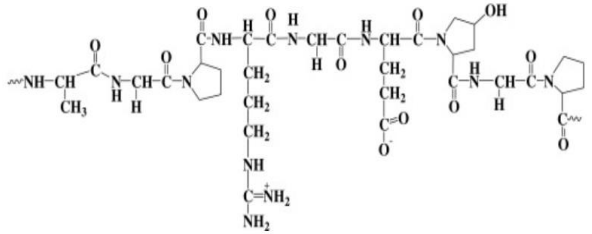
Film Production

Biodegradable Polymers:

Sodium Alginate (SA)



Gelatin (GN)



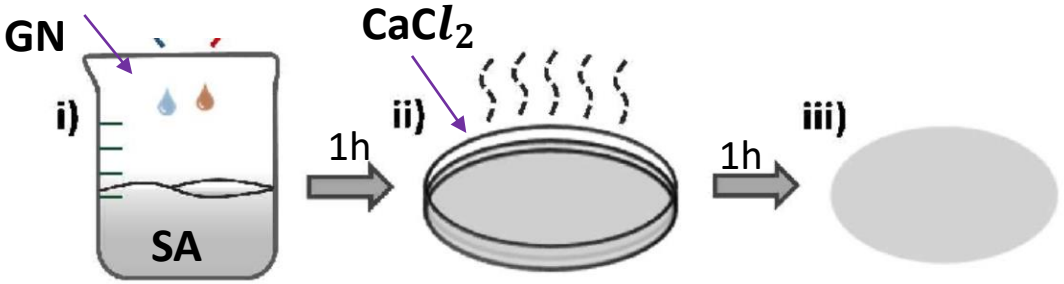
The solutions (SA and GN) were dissolved separately in distilled water at 50ºC for 1h and 3h, respectively.

Films:

SA 2% w/v + GN 1% w/v + dH_2O + CaCl_2 2% w/v + CS1

SA 2% w/v + GN 1% w/v + dH_2O + CaCl_2 2% w/v + CS4

SA 2% w/v + GN 1% w/v + dH_2O + CaCl_2 2% w/v - used as a control



Low stirring

4ºC overnight; Dried for 6 days at RT

Washed in dH_2O; 150 rpm; 15 min.

CLO-Loaded Microcapsules Morphology

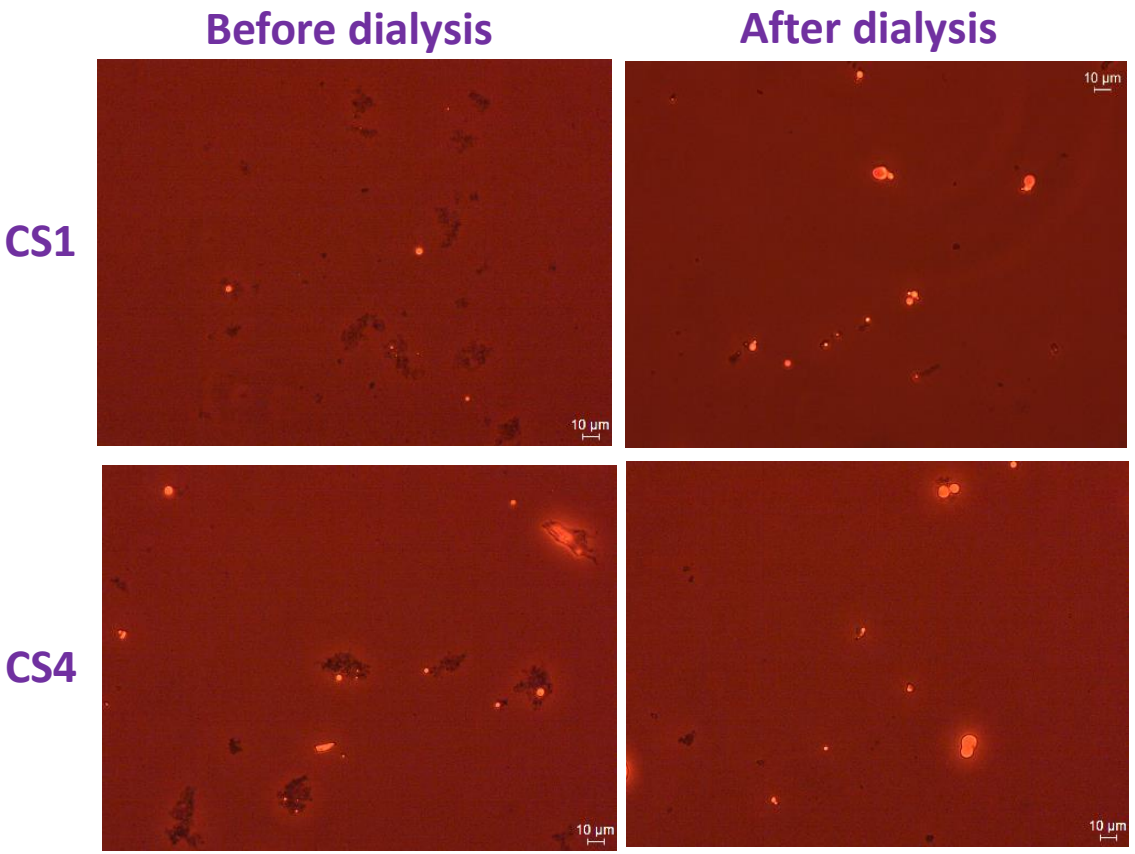
Technique:
Fluorescence microscopy;
40x magnification (20 µm scale bar)

Observations:

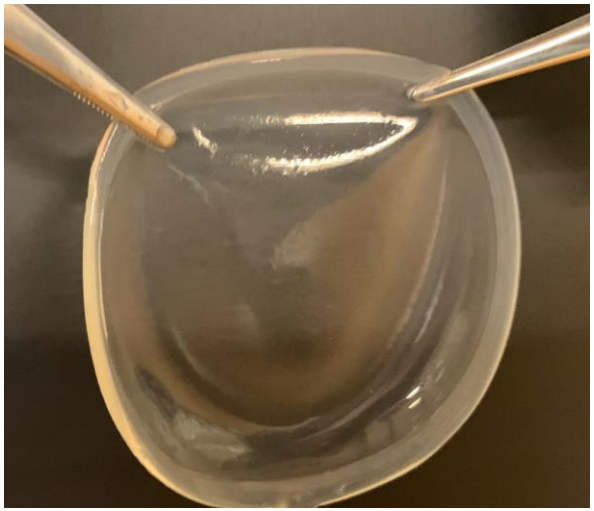
Elimination of the non-reacted polymer with dialysis.

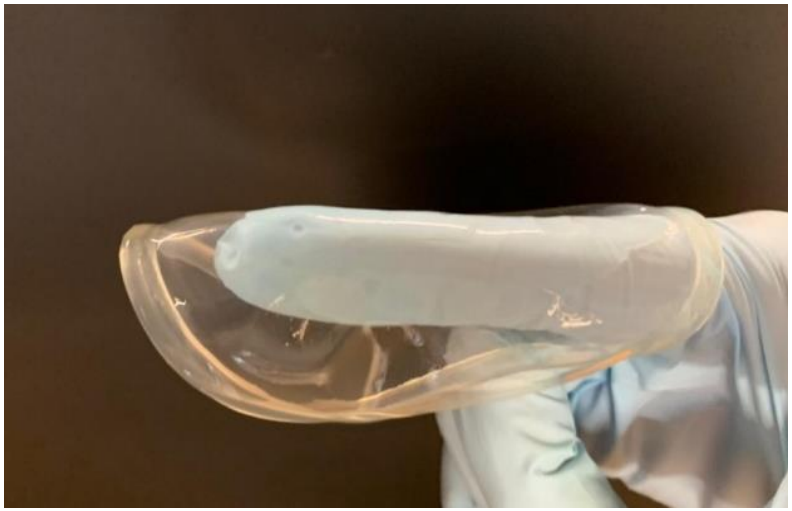
Greater microcapsule and unloaded chitosan aggregation on CS4 than on CS1.

Microcapsules produced with no pH adjustment were more successful.



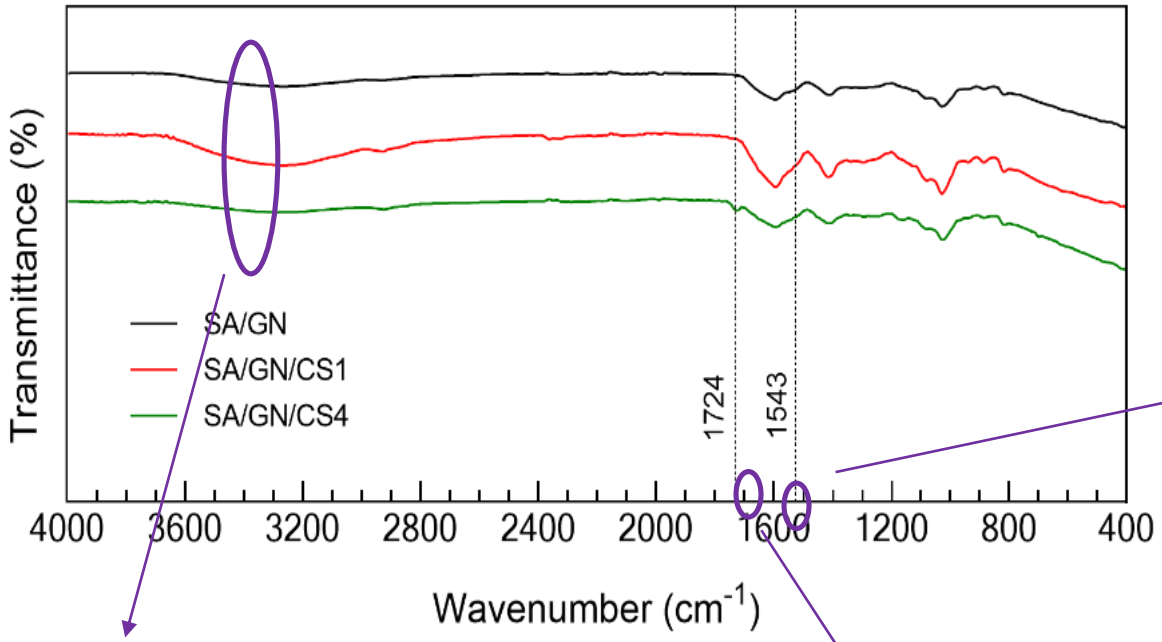
Films Morphology





- Very hydrated films;
- Flexible;
- Homogeneous;
- Regular distribution of microcapsules.

Chemical Characterization



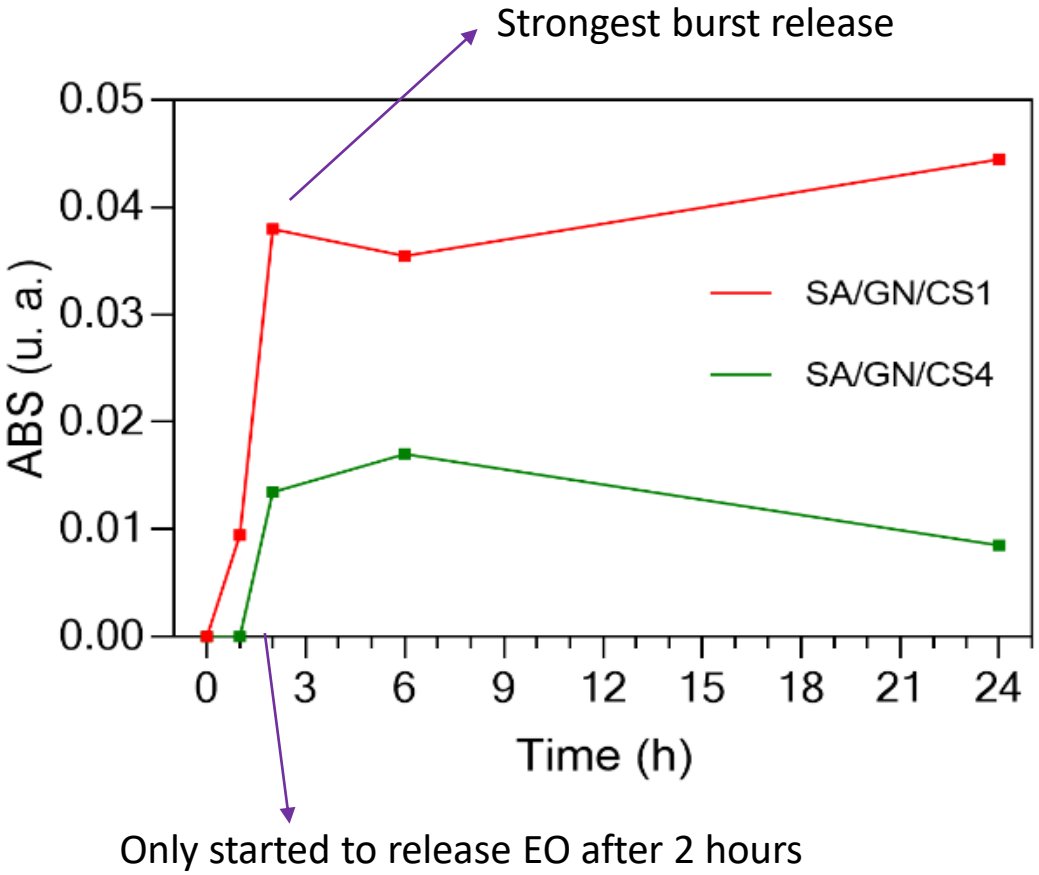
Aromatic ring C=C skeleton vibration of an aromatic substance

Hydrogen bonded O–H stretching vibrations

Presence of CLO → C=O stretching vibrations of the oil components

CLO was more easily noticeable on the CS4-containing films than on the CS1, which may indicate that the EOs molecules were larger

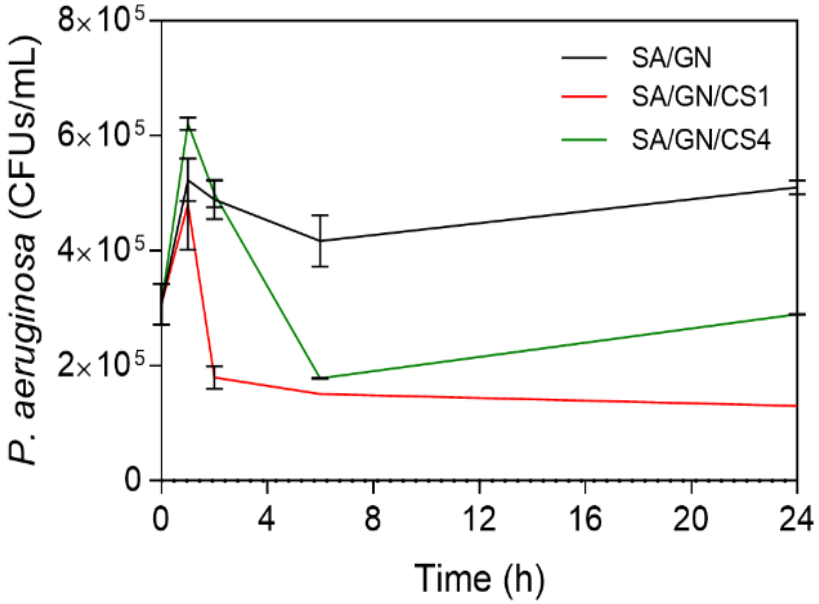
CLO Release Profile

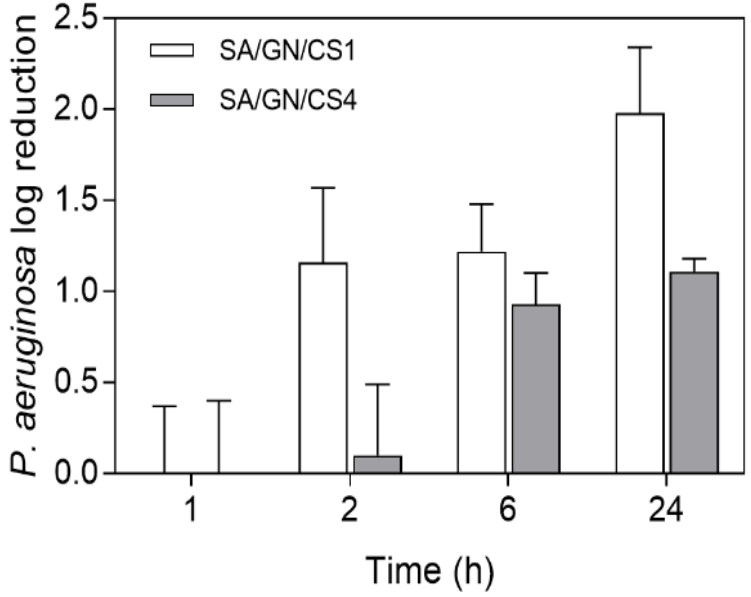


Lower release profile for the CS4-containing samples

Antimicrobial Action

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

2C2T Group

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