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S4. Coatings for Food Packaging and Preservation

Chitosan-based multicomponent antibacterial coatings to prevent biofilm formation for food packaging

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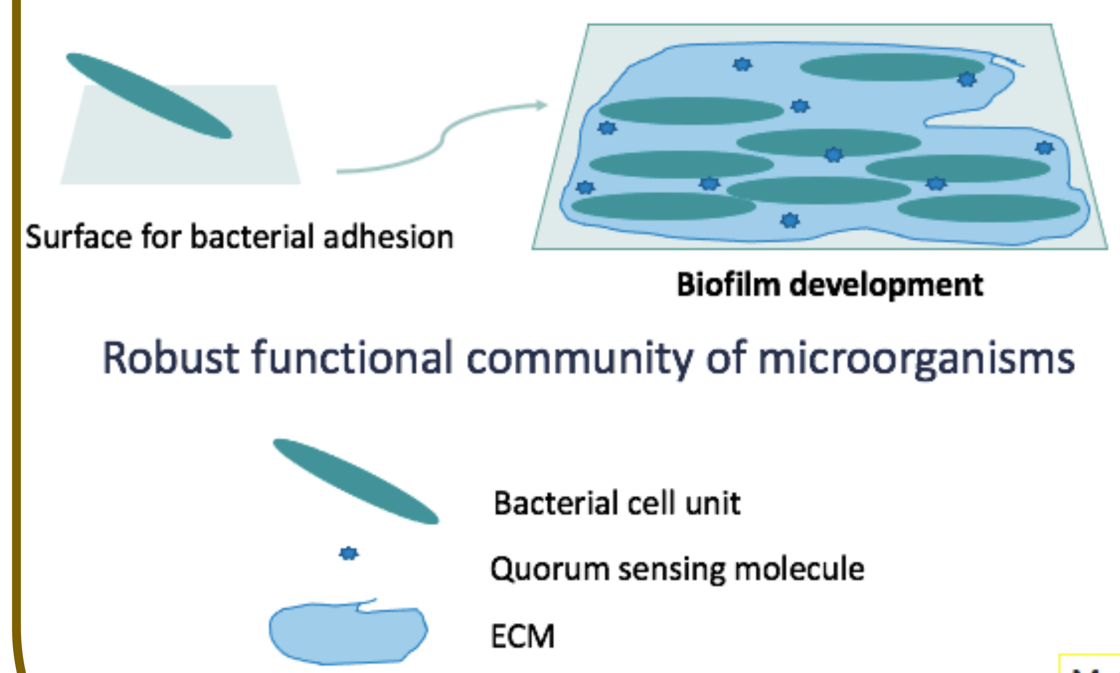
Introduction

The uncontrolled growth of biofilms and rise of antimicrobial resistance (AMR) cause significant damage in many industrial and public sectors, including food industry. Chitosan (CS), an abundant non-toxic polysaccharide, shows intrinsic antimicrobial activity against a wide range of pathogens and posses antibiofilm properties. CS and CS-based biomaterials of different forms can be effective additives in food and packaging industries to inhibit the formation of biofilms and prevent resistance development [1,2]. The application of inorganic nanophases, such as silver nanoparticles (AgNPs), has received attention due to their synergistic action upon combination with other antimicrobials [3]. Robust synergistic nanoantimicrobial (NAM) hybrid systems can be produced by combining CS and AgNPs. Further addition of the cross-linking agent with the intrinsic antimicrobial action, Tannic Acid (TA), brings mechanical robustness and more pronounced antipathogen effect. Here we produce bioactive CS/TA/AgNPs composite in a form of film or the coating to fight antimicrobial resistance and biofilm growth.

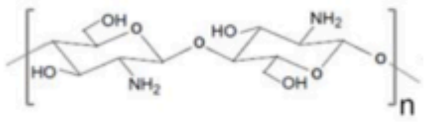
Aim of the project: Fighting biofilms

Biofilms are collective aggregates of bacteria of one or more types in which cells are embedded in a self-secreted extracellular polymeric matrix (ECM); grow on many different surfaces.

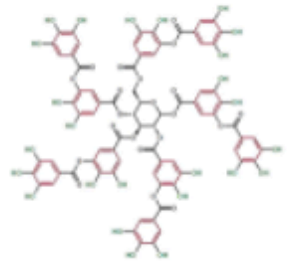


Chitosan

Cationic effect



Tannic Acid



Metal Nanoparticles

ROS and ion release

Figure 1. Scheme of biofilm development and examples of antimicrobials.

Methodology for production of hybrid CS-based composites

Films with various compositions:
Each **single component has intrinsic antimicrobial property** and preserves it upon combination with the other agents

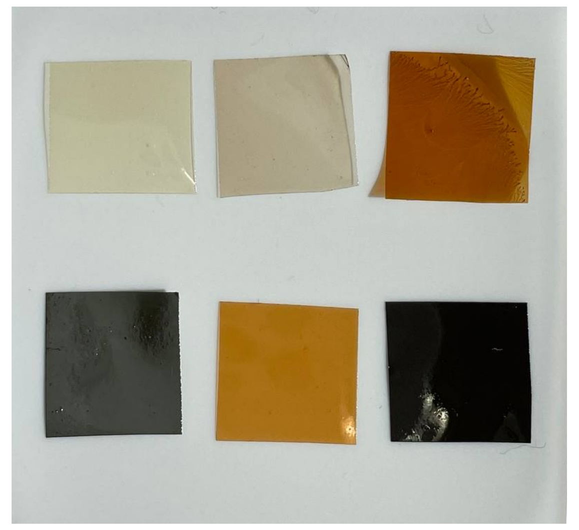


Figure 2. Photograph of self-standing CS-based films.

PROS

- ✓Green method
- ✓Fast enough
- ✓Easy scalable
- ✓Not expensive
- ✓Tunable properties

CONS

- Washing steps are needed
- Toxicity investigation

Characterization of:
- mother solution;
- films/coating;
- antimicrobial and antibiofilm tests.

3-step green wet-chemical process:

1. Reduction of $AgNO_3$ to AgNPs in presence of reducing agents (4 h, 65-75 °C);
2. Cross-linking;
3. Drying in the oven overnight (55 °C) in supported or unsupported manner by solution casting method.

CS-based films characterization: TEM and UV-vis of mother solutions

Morphology of as-synthesized AgNPs was investigated by **transmission electron microscopy (TEM)**. They appeared spherical and quite polydispersed (Fig. 3). Size analysis was performed on TEM images, and a mean diameter of 27 ± 17 nm was obtained.

UV-vis spectroscopy proved the successful formation of AgNPs by *in situ* synthesis (Fig. 4). Their stability was also investigated (1 week). Upon addition of GA crosslinking agent, the formation of AgNPs has been boosted. Stability over time was reached.

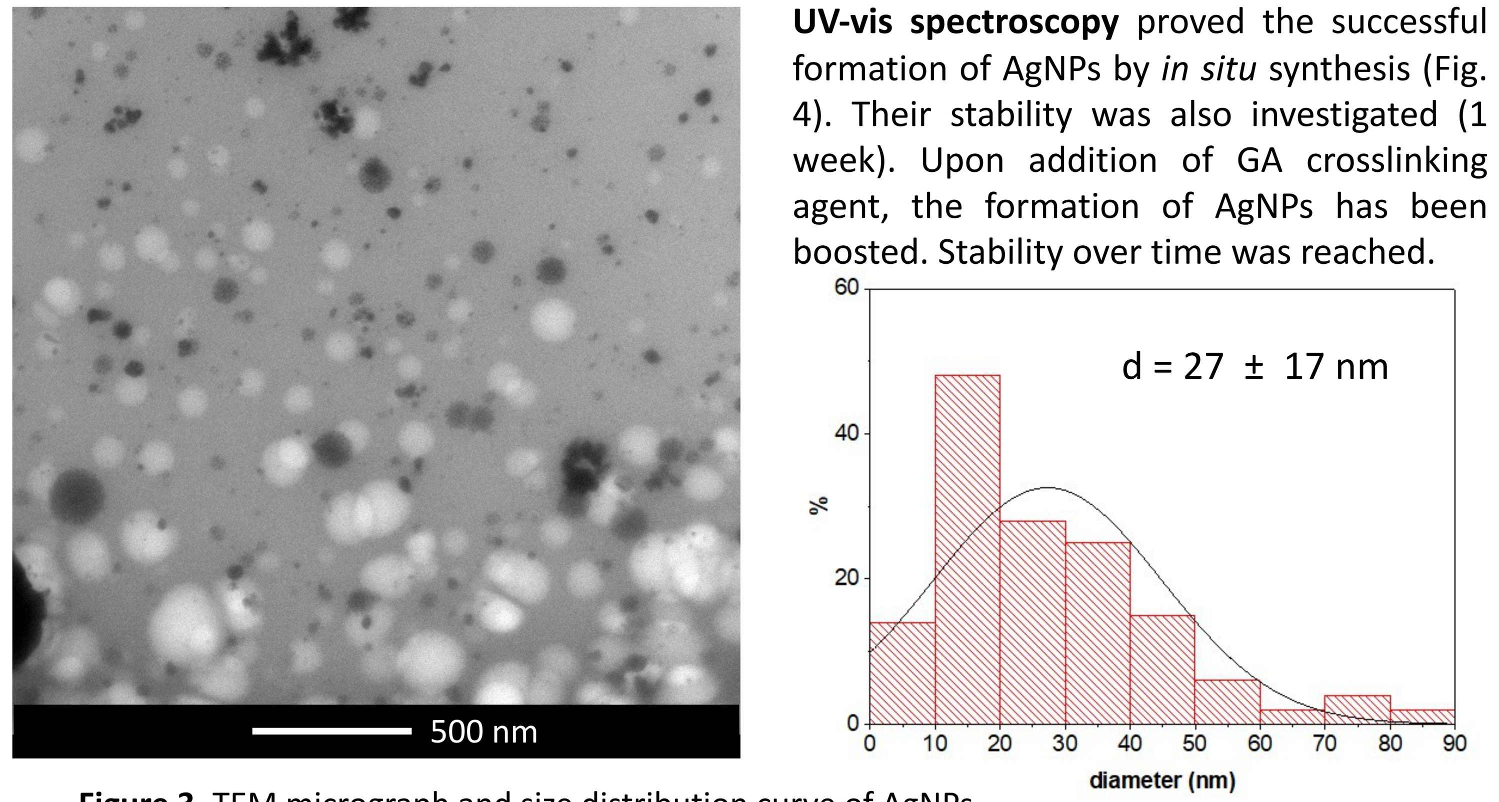


Figure 3. TEM micrograph and size distribution curve of AgNPs.

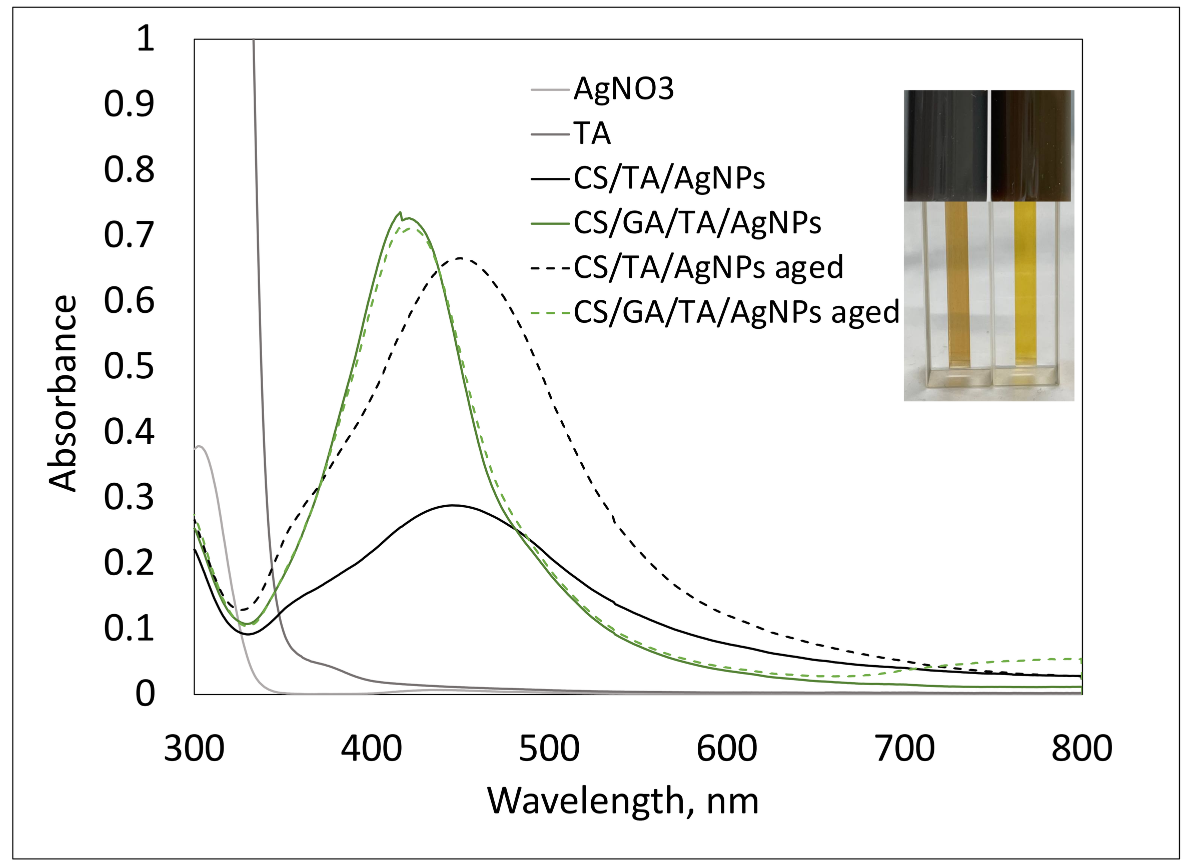


Figure 4. UV-vis spectra of mother solutions with different compositions

CS-based films characterization: FTIR and thickness measurements

The AgNPs complexation and CS/TA crosslinking was investigated by **Fourier transform infrared spectroscopy (FTIR)**. Band appearance at 825 cm^{-1} potentially corresponds to the interaction of the colloids with the oxygen-rich groups (Fig.6). Peak shifts occur due to coordination bond between AgNPs and electron rich groups of both CS and TA. We observe shifts corresponding to vibration of CO bond or N-H bending at 1710.2 cm^{-1} (to 1713.9 cm^{-1}); after addition of TA we see shift from 1638.9 cm^{-1} to 1627.8 cm^{-1}. Asymmetric vibration of CO or C-N bond changes wavenumber from 1252.8 cm^{-1} to 1246 cm^{-1}.

Due to these aspects, we can say that stabilization and cross-linking were successful.

Thickness on the films was manually measured (Fig. 5):

CS/AgNPs/TA ~40 µm
40 microns / 35 microns / 35 microns / 45 microns

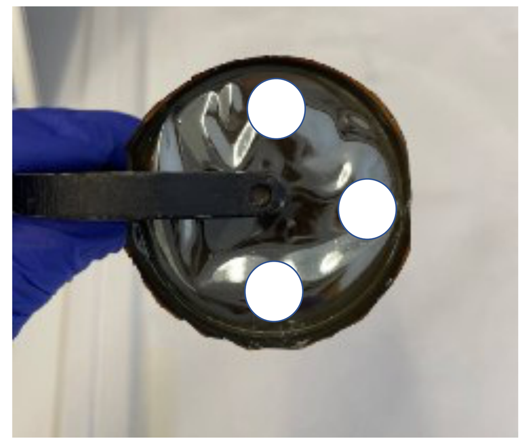


Figure 5. Points of data collection during thickness measurements.

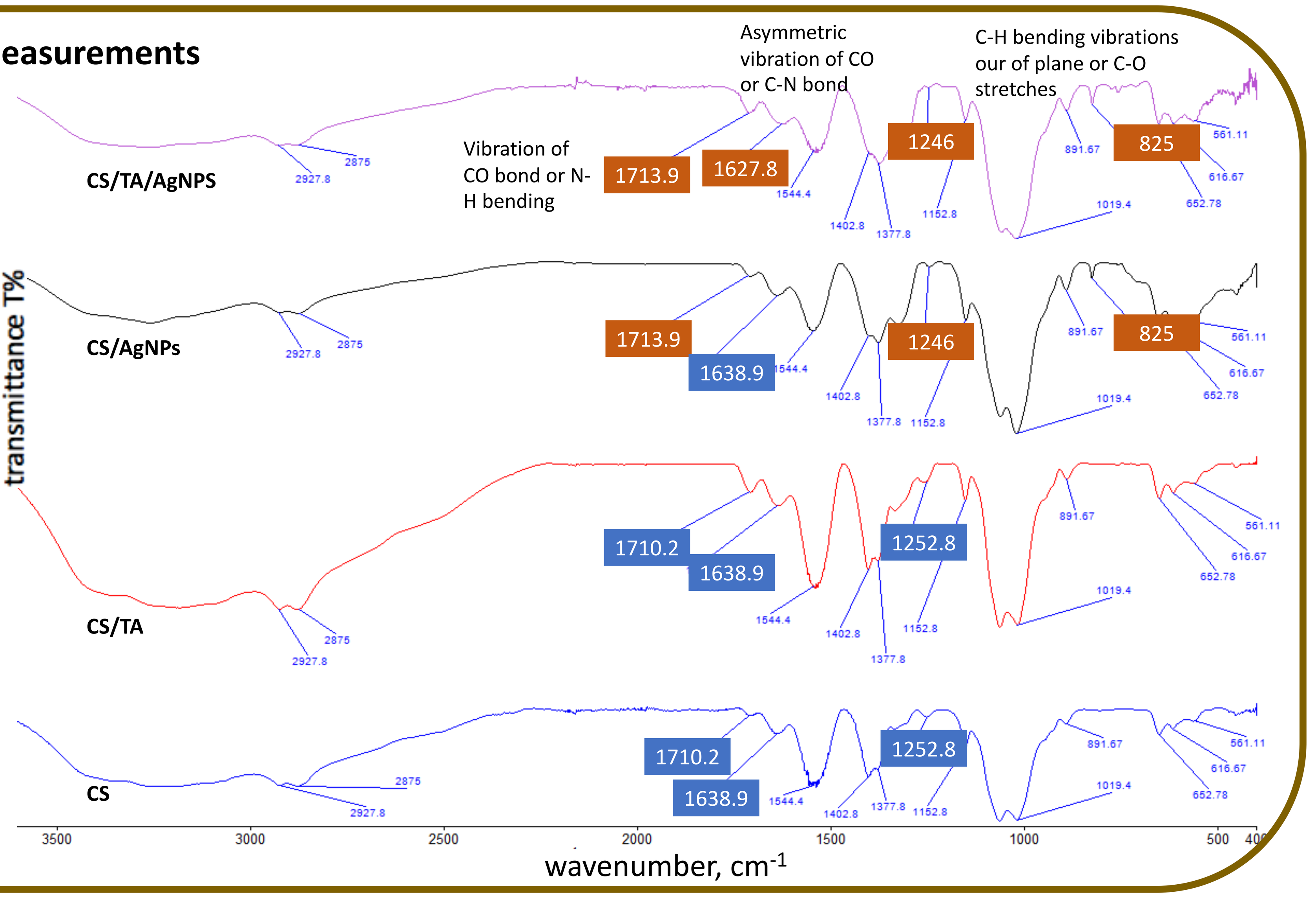


Figure 6 (on the right). FTIR spectra corresponding to CS-based films of different compositions with highlights on peak shifts and peak occurrence.

Conclusions

Upon combination of inorganic and organic phases, potential antibiofilm hybrid film or coating with enhanced activity were prepared by easily scalable green solution casting method. CS and TA were used as reducing and stabilizing agents for *in situ* production of AgNPs. Based on TEM and spectroscopic characterization, we concluded that synthesized particles have spherical shape and have mean diameter of ~30 nm. These synergistic films with complex composition are *potentially active* against a broad spectrum of pathogenic and resistant microorganisms, as well as biofilms. Optimization of synthetic parameters for production of mechanically robust films and effective coatings, characterization and biological activity tests are ongoing.

References

[1] H. Wang et al., J Agric Food Chem, 2018, 66(2), 395-413.
[2] B. Kaczmarek et al., Materials Letters, 2019, 245, 22-24.
[3] E.A. Kukushkina et al., Nanomaterials, 2021, 11(7), 1687.

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MARIE CURIE ACTIONS

BrEAKING BaD BIOFILMS

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