Synthesis of silver nanoparticles using orange peel extracts and their antimicrobial activity.

Willian M. da Silva (IC), Caio Barros (IC), Ljubica Tasic (PG)

Abstract
Silver nanoparticles (AgNP) can be synthesized using biological, chemical and physical methods and several kinds of reducing agents. In this project, the biological method was employed using orange peel extract as the reducing agent. In addition, the bactericidal activity of the biogenic AgNP against bacteria Xanthomonas axonopodis pv. citri will be evaluated.

Keywords: Biochemistry, Silver nanoparticles, Xanthomonas axonopodis pv. citri.

Introduction
Silver nanoparticles (AgNP) have been studied due to their antimicrobial, inhibitory and bactericidal activities against Gram-positive and Gram-negative bacteria. Despite the great number of methods by which AgNP can be synthesized, the biological method is used in this project for being considered environmentally friendly and low-cost since the plant extracts were used as reducing agents. The chemical and physical methods utilize synthesized chemical products that may generate harmful residues and are usually more expensive.1

The synthesis takes place with the reduction of the Ag+ ion to Ag0 (Silver metal). In this project, the reducing agents are proteins and biomolecules extracted from the orange peel and hesperidin. The characterization involves the determination of Zeta potential and size of the AgNP by DLS (Dynamic Light Scattering). Studies have shown that silver nanoparticles have antimicrobial activity against bacteria that causes citrus canker in citrus plants, known as Xanthomonas axonopodis pv. citri.2 Thus, this project also aims to evaluate the activity of biogenic AgNP synthesized by orange peel extract on strains of this bacterium, which causes great damage in the Brazilian citrusculture.

Results and Discussion
Orange peel extract and the isolated hesperidin were successful on inducing the formation of silver nanoparticles (AgNP).

It was observed by kinetics experiments performed on UV-visible spectroscopy that the hesperidin reduces the silver (I) nitrate to silver nanoparticles faster than the orange peel extract does. While the synthesis using hesperidin takes place in minutes, turning the color of the solution from pale yellow to orange and then brownish, the one using the extract only stabilizes after approximately 10 hours. The characterization of the nanoparticles produced is presented in the Chart 1.

Chart 1. Characterization of the synthesized AgNP

<table>
<thead>
<tr>
<th>Orange peel extract</th>
<th>Hesperidin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeta Potential (mV)</td>
<td>-19.9</td>
</tr>
<tr>
<td>Size (nm)</td>
<td>152.2</td>
</tr>
</tbody>
</table>

It is noticeable that AgNP produced by Hesperidin have a much lower Zeta Potential and are large, revealing the poor electrostatic and steric stabilization. On the other hand, the AgNP produced by the orange peel extract appear to be more stable due to the higher Zeta potential and being somewhat smaller particles.

Conclusions
Both methods for AgNP synthesis used were efficient, showing that hesperidin is indeed one of the reducing biomolecules in the orange peel extract. However, there are more reducing agents and stabilizing molecules involved in the biogenic AgNP synthesis. In the future, the antimicrobial activity of the AgNP produced will be analysed against strains of Xanthomonas.

Acknowledgement
Financial support from SEA-UNICAMP is acknowledged and also UNICAMP for the infrastructure and facilities used in this research.

References
2 Ballotin, D. P. M. Caracterização de nanopartículas de prata e sua aplicação na produção de tecidos antimicrobianos; PhD Thesis, Instituto de Química, UNICAMP, Campinas, 2014.