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Green Production of Chitosan Nanoparticles and their Potential as Antimicrobial Agents

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Abstract : The application of nanoscale materials and nanostructures is an emerging area, these since materials may provide solutions to technological and environmental challenges in order to preserve the environment and natural resources. To reach this goal, the increasing demand must be accompanied by 'green' synthesis methods. Chitosan is a natural, nontoxic, biopolymer derived by the deacetylation of chitin and has great potential for a wide range of applications in the biological and biomedical areas, due to its biodegradability, biocompatibility, non-toxicity and versatile chemical and physical properties. Chitosan also presents high antimicrobial activities against a wide variety of pathogenic and spoilage microorganisms. Ultrasonication is a common tool for the preparation and processing of polymer nanoparticles. It is particularly effective in breaking up aggregates and in reducing the size and polydispersity of nanoparticles. High-intensity ultrasonication has the potential to modify chitosan molecular weight and, thus, alter or improve chitosan functional properties. The aim of this study was to evaluate the influence of sonication intensity and time on the changes of commercial chitosan characteristics, such as molecular weight and its potential antibacterial activity against Gram-negative bacteria. The nanoparticles (NPs) were produced from two commercial chitosans, of medium molecular weight (CS-MMW) and low molecular weight (CS-LMW) from Sigma-Aldrich®. These samples (2%) were solubilized in 100 mM sodium acetate pH 4.0, placed on ice and irradiated with an ultrasound SONIC ultrasonic probe (model 750 W), equipped with a 1/2" microtip during 30 min at 4°C. It was used on constant duty cycle and 40% amplitude with 1/1s intervals. The ultrasonic degradation of CS-MMW and CS-LMW were followed up by means of ζ-potential (Brookhaven Instruments, model 90Plus) and dynamic light scattering (DLS) measurements. After sonication, the concentrated samples were diluted 100 times and placed in fluorescence quartz cuvettes (Hellma 111-QS, 10 mm light path). The distributions of the colloidal particles were calculated from the DLS and ζ-potential are measurements taken for the CS-MMW and CS-LMW solutions before and after (CS-MMW30 and CS-LMW30) sonication for 30 min. Regarding the results for the chitosan sample, the major bands can be distinguished centered at Radius hydrodynamic (Rh), showed different distributions for CS-MMW (Rh=690.0 nm, ζ=26.52±2.4), CS-LMW (Rh=607.4 and 2805.4 nm, ζ=24.51±1.29), CS-MMW30 (Rh=201.5 and 1064.1 nm, ζ=24.78±2.4) and CS-LMW30 (Rh=492.5, ζ=26.12±0.85). The minimal inhibitory concentration (MIC) was determined using different chitosan samples concentrations. MIC values were determined against to E. coli (106 cells) harvested from an LB medium (Luria-Bertani BD™) after 18h growth at 37 ºC. Subsequently, the cell suspension was serially diluted in saline solution (0.8% NaCl) and plated on solid LB at 37°C for 18 h. Colony-forming units were counted. The samples showed different MICs against E. coli for CS-LMW (1.5mg), CS-MMW30 (1.5 mg/mL) and CS-LMW30 (1.0 mg/mL). The results demonstrate that the production of nanoparticles by modification of their molecular weight by ultrasonication is simple to be performed and dispense acid solvent addition. Molecular weight modifications are enough to provoke changes in the antimicrobial potential of the nanoparticles produced in this way.

Keywords : antimicrobial agent, chitosan, green production, nanoparticles
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