Scientific Report *on the*
implementation of the project
"Valorization of Chitinous Material from Recyclable Waste by Using It in a Some Potential Biological Applications (ReWaChi)"
Financing contract 70/2021,
Project code PN-III-P4-ID-PCE-2020-2243
Period: January 3rd, 2022 – December 31st, 2022

Establishing optimal chitosan formulations to achieve the best response of biological systems

Abstract

The study conducted this year led to the following conclusions:

- It is noted that in terms of the concentration of NaOH and the deacetylation duration, the conditions for obtaining the maximum values for both the degree of deacetylation and the molar mass are very close. The solid liquid ratio, which otherwise slightly influences these two characteristics of chitosan, appears to be favourable at lower values (15/1) for the increase in the degree of deacetylation, while the maximum molar mass can be obtained at a ratio of 18/1. Depending on the relative importance of the two criteria (degree of deacetylation and molar mass) appropriate optimum points can be selected from the Pareto front, for which the operating conditions can be identified.
- Chitosan manifests similar effects in *in vivo* systems at the physiological level, especially by blocking membrane activity. The effects are influenced by the properties of particles in the solutions as well as the ratio of chitin: chitosan: oligochitosan, which influences the passage through the membranes.
- Toxicity or cytotoxicity in most of the tested solutions is reduced or moderate in *Artemia*. Testing on more complex organisms *G. balcanicus*, denotes another aspect, namely, the speed of penetration of the polymer at the gill level depends on the ratio chitin : chitosan and generates the modification of ionic hemostasis and the decrease in viability, in a short time. The presence of oligochitosan favors the survival of the larvae, the penetration of oligochitosan being favored by ingestion.
- Evaluating with MTT the impact of chitosan molecules taken in the study on cell viability revealed the induction of a cytotoxic effect, differentiated in amplitude depending on the compound, the dose of *in vitro* treatment – the existence of the dose-effect relationship being demonstrated – and the type of cell culture. It is worth noting that in tumor cell cultures HeLa the degree of damage to cell viability is more pronounced than that of healthy cells MCF-12A. Correlated with the results obtained by the MTT test, the impairment of the morphology and, implicitly, of the cellular viability, was more intense in the case of HeLa tumor cells, after the 48-hour treatment.
- Chitosan passes through the digestive tract becoming microscopically highlighted in epithelial cells, cuticle, cells of the digestive tract, as well as in other types of cells such as myocytes, after 48 hours of exposing the organisms to concentrations of at least 35 μg/mL Cs, the FITC marking method being effective for identifying chitosan in cells.
- All the results relating to the evaluation of the expression levels of chitinase-like proteins, in particular YKL 40 in normal and tumor cell lines, converge to the conclusion that chitosan-based formulas have induced dose-dependent cellular reactivity, demonstrating the existence of the dose-effect relationship. Also, the cytophysiological response was influenced by both the duration of the treatment and the type of cell culture, being more intense after 48 treatment and on the tumor cell line.
- The NP properties of chitosan, such as size, polydispersity and zeta potential, depend both on the molecular weight and degree of deacetylation of chitosan, as well as on the surfactant used.
- The antioxidant activity of the chitosan sample was achieved by monitoring the capture capacity of two types of radicals: ROS (short-lived radicals) by chemiluminescence method and cationic long-lived radicals $ABTS^{\cdot +}$ by the TEAC method. Chitosan samples showed ROS radical capture activity between 55.1-98.4% and an inhibition capacity of ABTS radicals$^{\cdot +}$ between 14,33 and 90,9 %, the capacity being influenced by the degree of deacetylation and the molecular weight of chitosan.

Summary of progress stages 1 and 2
Deliverables made: 2 published articles; 1 article in evaluation
Dissemination of results: 2 published articles, 1 article in evaluation and 11 presentations at scientific events
Result indicators: 1 + 2 = 3 scientific articles – achieved