# Self-assembly levan, as natural active bio-nanocarrier - new approach in technology

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## Introduction

To find a material that enables the encapsulation of hydrophobic substances, can penetrate the skin, and has a natural origin at the same time, is a big challenge. Therefore, the search for such materials obtained in green chemistry processes is still ongoing. Biopolymers are biodegradable and biocompatible molecules, and their physicochemical properties and biological activity attract the attention of not only scientists, but also industry (Verma et al. 2020). This group includes polysaccharides. Their use is dictated by their various properties.

Levan is a fructose polymer, obtained both from plant and from microbiological processes (Domżał-Kędzia et al. 2019). It is a compound characterized by many interesting functional features in the context of its use in industry. Levan can self-assemble in water, which makes it an extremely interesting raw material (Ağçeli et al. 2020). Traditional microbiological preparation of levan not only generates considerable costs, but also has a large impact on the environment. To meet industrial and environmental expectations and obtain the most eco-friendly materials, more and more new technological solutions are sought, which are then verified in terms of their impact on the environment. Life Cycle Assessment allows the identification of the most critical points in each process, and helps find a greener solution.

Two processes for the preparation of levan nanoparticles have been developed, including one that is waste-free. Levan nanoparticles were obtained in two different ways - the first one involves obtaining them from a previously precipitated polymer, and the second obtains them directly from the post-culture supernatant. The nanoparticles were compared with each other in terms of their surface area, size and anti-radical properties. The ability of nanoparticles to penetrate the skin and their effect on the skin were also investigated.

## Materials and methods

Two processes for the preparation of levan nanoparticles were used in the research - one involving the precipitation of the polymer from the substrate and the other, aiming for the lowest possible consumption of materials and minimized amount of waste, using membrane filtration. *B. subtilis* bacteria were cultured in a 5 L bioreactor in a medium containing sucrose. The supernatant obtained after fermentation was divided in half - in one half the polymer was precipitated and redissolved, while in the other it was left as a source of nanoparticles.

The nanoparticles obtained in the two processes were subjected to physicochemical and biological analysis in terms of their industrial utility. Part of the precipitated polymer was subjected to spectroscopic examination (1H NMR, IR) to confirm its structure and to determine CAC (Critical Aggregation Concentration) value. The molecular weight and its distribution were determined by GPC (Gel Permeation Chromatography). Size, polydispersity and Zeta potential were determined by DLS (Dynamic Light Scattering) analysis. The stability of the obtained nanoparticles was determined 1 month after their preparation. The surface structure of the nanoparticles was also investigated with TEM (Transmission electron microscopy) analysis. The antiradical abilities of the nanoparticles obtained by both processes were also determined. In a further part of the research work, a fluorescent dye was encapsulated, and then experiments were conducted to verify the ability of the nanoparticles to penetrate through the stratum corneum layer, and their effect on the skin. This part of the experiments was performed using the Franz diffusion cell, CLSM (Confocal Laser Scanning Microscopy) and a confocal Raman microscope.

## Results

Spectroscopic analyses confirmed the levan production during *B. subtilis* KB1 fermentation. Levan nanoparticles were obtained in two processes and analysed. GPC analysis determined the bimodal molecular

weight distribution of the polymer, which is characteristic of levan obtained from *B. subtilis*. The molecular weight was 2710 and 11.35 kDa. The levan nanoparticles obtained by both processes had similar size, polydispersity and Zeta potential. These parameters were respectively 214.1 - 245.88 nm, PdI 0.12 - 0.14 and (-4.05) - (-5.08) mV. The nanoparticles of the precipitated polymer were slightly smaller. The differences in the surface structure and antiradical abilities between the levan nanoparticles obtained in the two processes were determined. The surface of the levan nanoparticles obtained after dissolving the polymer is quite regular and spherical, while the surface of the nanoparticles obtained directly from the supernatant was rather irregular. It was shown that the nanoparticles derived directly from the post-culture supernatant exhibit greater antiradical activity. It was determined that the levan nanoparticles penetrate the stratum corneum and affect the condition of the skin.

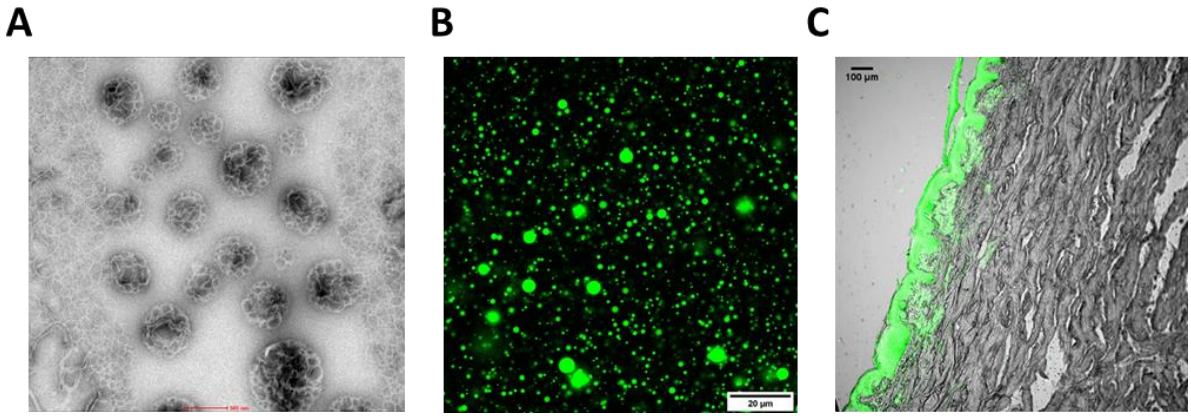


Fig.1. Levan nanoparticles: A) TEM analysis, B) CLSM with the encapsulated fluorescent dye, C) penetration in the skin.

**Bibliography**

**1.** Verma M.L et al (2020) 'Chapter 5 - Microbial production of biopolymers with potential biotechnological applications, Editor(s): Kunal Pal, Indranil Banerjee, Preetam Sarkar, Doman Kim, Win-Ping Deng, Navneet Kumar Dubey, Kaustav Majumder, Biopolymer-Based Formulations, Pp.105-137, https://doi.org/10.1016/B978-0-12-816897-4.00005-9.
2. Domżał-Kędzia, M. et al. (2019) 'Fermentation parameters and conditions affecting levan production and its potential applications in cosmetics', Bioorganic Chemistry), pp. 1–8. doi: 10.1016/j.bioorg.2019.02.012.
3. Ağçeli G.K. et al (2020). 'Nano-sized biopolymer levan: Its antimicrobial, anti-biofilm and anti-cancer effects', Carbohydrate Research, Vol 494, 108068, https://doi.org/10.1016/j.carres.2020.108068

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