# 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 & 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 ($^1$H 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 (Fig.1A). 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 (Fig.1B).

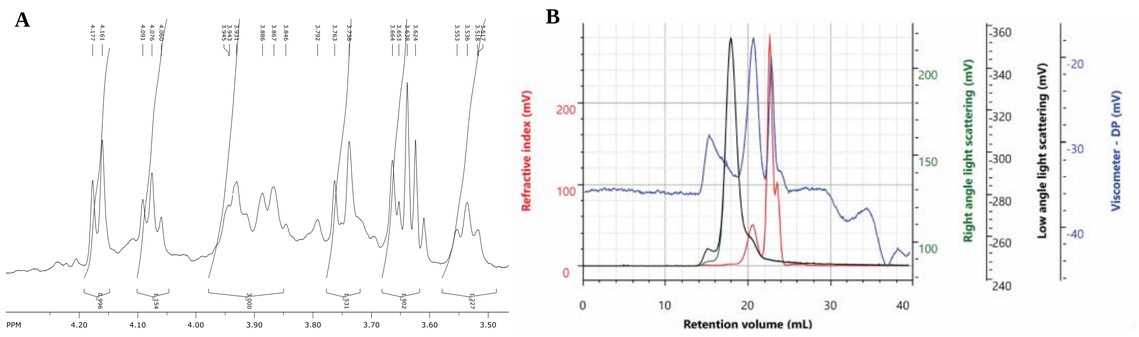


**Fig.1. A) $^1$H NMR spectrum of levan, B) Chromatogram of levan.**

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 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 (Fig.2).

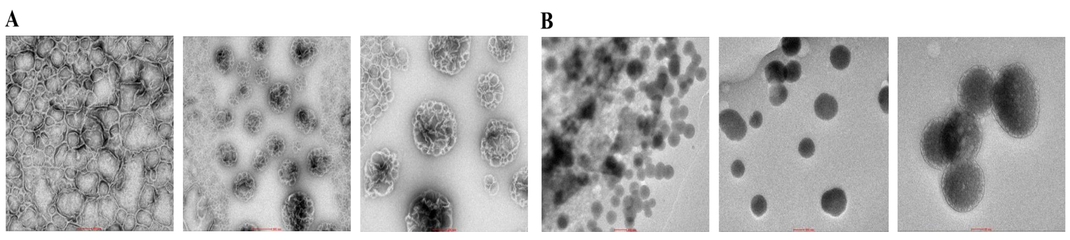


**Fig.2. TEM analysis of levan nanoparticles A) directly from the supernatant, B) from levan precipitated with ethanol.**

The levan nanoparticles obtained directly was subjected to stability tests. The results shown that nanoparticles were stable for 1 month in room temperature.

**Table 1. Stability studies of levan nanoparticles.**

| Sample | Z-Ave [d.nm] | PdI | ZP [mV] |
|---|---|---|---|
| Supernatant day 0 | 245.88 ± 16.09 | 0.140 ± 0.024 | -5.08 ± 1.82 |
| Day 7th | 225.5 ± 3.30 | 0.188 ± 0.020 | -1.66 ± 0.41 |
| Day 30th | 237.8 ± 2.5 | 0.121 ± 0.027 | 0.51 ± 0.20 |

It was shown that the nanoparticles derived directly from the post-culture supernatant exhibit greater antiradical activity (Fig. 4). To determine the penetration of levan nanoparticles in the skin, fluorescent dyes were encapsulated – coumarin and Nile red (Fig. 3). It was determined that the levan nanoparticles penetrate the *stratum corneum* and affect the condition of the skin.

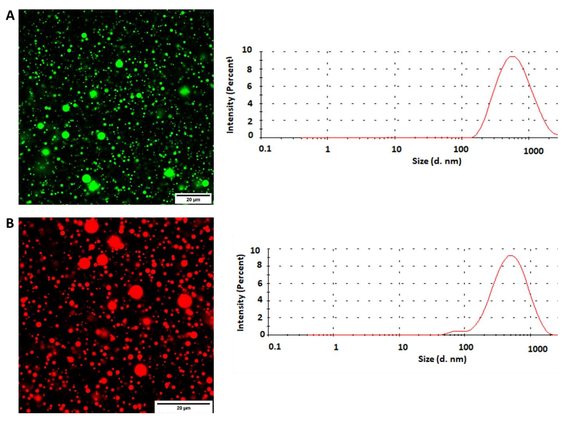


**Fig. 3. Levan nanoparticles with coumarin A) and B) Nile red - confocal microscope and DLS analysis.**

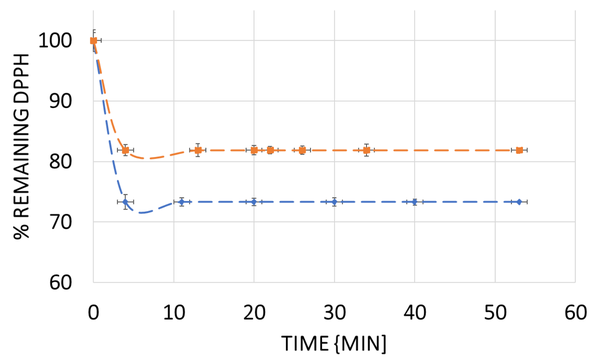


**Fig. 4. Antiradical properties of levan nanoparticles from precipitated polymer (orange) and from post-culture liquid (blue).**

Levan nanoparticles are able to penetrate through the *stratum corneum*. The penetration was evaluated after 1, 4 and 8 h after applying the nanoparticles solution on skin (Fig. 5). Raman spectroscopy shown that levan nanoparticles obtained from previously precipitated allowed to determine that nanoparticles obtained directly from the post-culture liquid are able to hold more water in the skin and in effect increases the moisturizing effect (Fig. 6). It was determined on the basis of the intensity of the water band at approx. 3200 cm$^{-1}$. The greater the signal intensity, the more water in the sample.

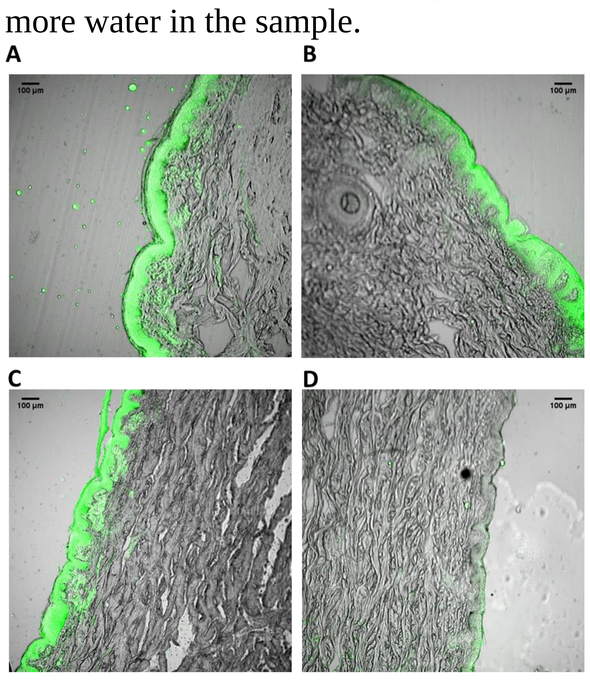


**Fig. 5. Penetration of levan nanoparticles with the Nile red in the skin after A) 1 h, B) 4 h, and C) 8 h, D) control.**

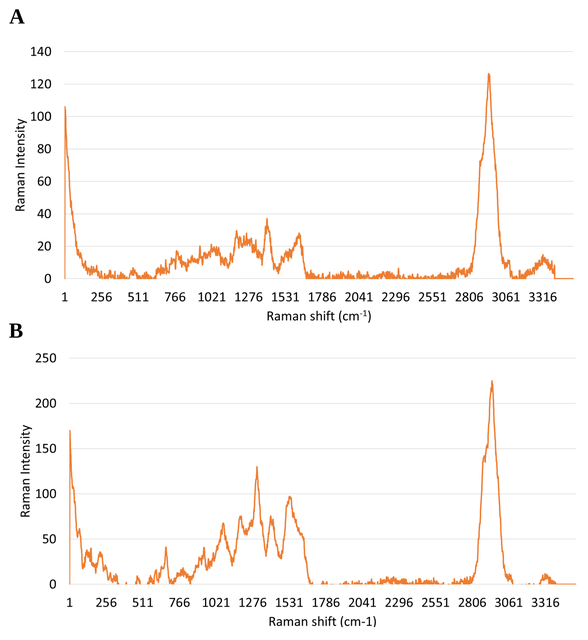


**Fig. 6. Raman spectra of skin treated with levan nanoparticles obtained A) directly from the post-culture liquid. B) from precipitated polymer.**

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