Nanostructured biopolymers, bionanofabrication and the path towards a sustainable nanotechnology

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Objectives and novelty

This doctoral thesis addresses the need for greener practices in nanotechnology by focusing on nanostructured biopolymers (NBs), a class of biopolymers characterized by at least one dimension in the nanoscale. This nanoscale size imparts enhanced and often novel properties to these nanomaterials compared to their bulk counterparts, such as high surface area, tunable surface chemistry, biodegradability, and mechanical robustness, making them attractive for sustainable applications. The main objective was to develop and investigate sustainable and reproducible approaches to synthesize NBs through both top-down and bottom-up routes, to characterize thoroughly, and to exploit their function as emulsifiers and aqueous dispersants for unidimensional carbon nanomaterials (1D CNMs), such as single-walled and multi-walled carbon nanotubes (SWCNTs, MWCNTs), and carbon nanofibers (CNFs), enabling practical devices.

The novelty of this work lies in: i) the development of reproducible top-down protocols for cellulose nanocrystals (CNCs), chitin nanocrystals (ChNCs), and silk fibroin nanofibers (SFNFs), including the optimization of CNC allomorph control via one-pot H_2SO_4 hydrolysis; ii) development of a rapid, non-destructive method based on dynamic light scattering (DLS) for CNC allomorph identification; iii) Application of NBs to stabilize oil-in-water Pickering emulsions and to disperse 1D CNMs in aqueous media without the use of surfactants or organic solvents, enabling the fabrication of conductive films, thermoelectric textiles, and electrochemical sensors; iv) bionanofabrication strategies through the bottom-up synthesis of bacterial nanocellulose (BNC), exploring alternative media, *in situ* nanomaterial additives, and emulsion templating to tailor porosity and introduce new functionalities.

Results

The one-pot H_2SO_4 hydrolysis was optimized to produce CNCs in both allomorphs (type I and type II), enabling control over the allomorph outcome while maximizing yield and reproducibility. The acid–cellulose contact time emerged as the key parameter governing the crystalline form. Additionally, dynamic light scattering (DLS) was validated as a rapid, non-destructive screening method to identify the allomorph outcome. Protocols were also established for ChNCs via HCl hydrolysis and for SFNFs via a water–$CaCl_2$–ethanol ternary solvent. X-ray diffraction, thermogravimetry, and transmission electron microscopy analyses confirmed high crystallinity and stability of the NBs produced by top-down routes (CNCs, ChNCs, and SFNFs).

As a proof of concept, oil-in-water Pickering emulsions were formulated and systematically compared across different oil/water ratios using each NB and compared for the first time using static light scattering and the visual determination of the emulsion stability over time. The analysis linked emulsion stability, droplet morphology, and interfacial behavior to NB structure. Type I CNCs stabilized emulsions best at 30/70 oil/water ratio, while SFNFs performed better at higher oil content. These results position NBs as sustainable emulsifiers capable of replacing conventional surfactants in selected regimes.

Aqueous dispersions of 1D CNMs (SWCNTs, MWCNTs and CNFs) were prepared and optimized using the prepared NBs. The objective was to achieve stable colloidal systems with high CNM concentrations, avoiding the use of organic solvents or dispersants. Among the NBs tested, type II CNCs consistently outperformed type I CNCs and ChNCs, displaying higher affinity and stabilization efficiency across all CNMs. ChNCs enabled stable dispersions with higher CNM content than type I CNCs. In contrast, SFNFs exhibited a distinct behavior: they were particularly effective in dispersing pristine CNTs but showed limited performance with CNFs.

The CNM/NB dispersions were extensively characterized to assess colloidal stability, structure, and surface chemistry. Selected formulations were used as water-based inks to fabricate conductive films via drop-casting and spray-coating. Electrical and electrochemical characterizations revealed competitive performance, especially for type II CNC-based films, which exhibited lower surface resistance and enhanced electrochemical response. Additionally, post-deposition thermal treatment improved the conductivity by 1 to 5 orders of magnitude, particularly in films with initially higher resistance. These results validate the use of NBs as green and effective dispersants for CNMs in water, enabling the scalable fabrication of conductive films without the need for hazardous chemicals.

Furthermore, the inks were tested in two proof-of-concept applications: thermoelectric textiles and electrochemical sensors. Prototype e-textiles were fabricated by dip-coating cotton fabrics with CNF/type II CNC inks and evaluated in terms of electrical conductivity, thermoelectric performance, and laundering resistance, confirming their feasibility for flexible and sustainable energy harvesting. Modified electrodes enabled the detection of two emerging contaminants in water, demonstrating the potential of these inks for environmental monitoring using greener fabrication methodologies.

Lastly, BNC was investigated as a bottom-up NB produced by bacterial synthesis, with strategies developed to tailor its structure and functionality through culture media optimization, incorporation of in situ nanomaterial additives (SWCNTs, carbon dots, and ChNCs), and oil-in-water emulsion templating. This approach enabled the fabrication of porous BNC composites with tunable porosity, controlled by emulsion composition and droplet size. These results consolidate bionanofabrication as a promising strategy for sustainable nanomaterial production, exploiting the inherent capabilities of living systems to conduct nanoscale synthesis.

Conclusions

This thesis demonstrates significant progress in sustainable nanotechnology through the development of reproducible and optimized synthesis protocols for nanostructured biopolymers (CNCs, ChNCs, SFNFs and BNC) and their validation in different applications. Using water as the sole processing medium, the work enables the dispersion of 1D-CNMs without organic solvents and surfactants, preserving their intrinsic properties and allowing the fabrication of functional inks for conductive films, thermoelectric textiles, and electrochemical sensors. The results highlight the excellent ability of NBs as aqueous dispersants, to stabilize Pickering emulsions under selected conditions, and the versatility of bionanofabrication strategies to tailor BNC composites.

These findings help balance sustainability with functionality, while opening new avenues for practical implementation of these green nanomaterials in energy and environmental technologies. Nevertheless, challenges remain, including scalability, reproducibility, and performance optimization at industrially relevant throughputs. Future work should refine NB synthesis and processing windows, broaden application domains and device integration, and incorporate sustainability assessments to quantify environmental benefits. Overall, this work consolidates nanostructured biopolymers as key enablers of green nanotechnology, emphasizing the importance of interdisciplinary collaboration to drive advancements in this field and highlighting the potential of NBs for applications in energy, sensing, and environmental remediation.

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Related publications

[1] Álvarez-Sánchez M.A., Calvo V., Carrera C., Colom E., Güemes L., Martínez-Barón C., González-Domínguez J.M. Emerging carbon nanotechnology. Advances and Thoughts from a Young Perspective. Bol. del Grupo Español del Carbón. 2021, 60, 8-19.

[2] Calvo V., González-Domínguez J.M., Benito A.M., Maser W.K. Synthesis and Processing of Nanomaterials Mediated by Living Organisms. Angewandte Chemie International Edition. 2022, 61, e202113286.

[3] Calvo V., Álvarez-Sánchez M.A., Güemes L., Martínez-Barón C., Baúlde S., Criado A., González-Domínguez J.M., Maser W.K, Benito A.M. Preparation of Cellulose Nanocrystals: Controlling the Crystalline Type by One-Pot Acid Hydrolysis. ACS Macro Letters. 2023, 12, 152-158.

[4] Calvo V., Fuentes L., Berdejo D., González-Domínguez J.M., Maser W.K., Benito A.M. Oil-in-Water Pickering Emulsions Stabilized with Nanostructured Biopolymers: A Venue for Templating Bacterial Cellulose. International Journal of Molecular Sciences. 2023, 24-17, 13141.

[5] Fuentes L., Calvo V., Berdejo D., González-Domínguez J.M., Maser W.K., Benito A.M. Bionanofabricación como Principio Sostenible de Obtención de Nanocelulosa Bacteriana. Boletín del Grupo Español del Carbón. 2023, 68, 11-14.

[6] Vázquez-Conejo B., Martínez-Barón C., Calvo V., García-Bordeje E., Ansón-Casaos A., González-Domínguez J.M., Maser W.K., Benito A.M. Nanoestructuras de Carbono Unidimensionales: Procesado Sostenible en Agua a Través de Nanocristales de Celulosa. Boletín del Grupo Español del Carbón. 2023, 68, 6-10.

[7] Calvo V., Paleo A.J., González-Domínguez J.M., Muñoz E., Krause B, Pötschke P., Maser W.K., Benito A.M. The Aqueous Processing of Carbon Nanofibers Via Cellulose Nanocrystals as a Green Path Towards e-Textiles with N-Type Thermoelectric Behaviour. Carbon. 2024, 217, 118640.

[8] Calvo V., Martínez-Barón C., Vázquez-Conejo B., Dominguez-Alfaro A., Paleo A.J., Villacampa B., Ansón-Casaos A., Maser W.K., Benito A.M., González-Domínguez J.M. Carbon Nanomaterials-Based Inks and Electrodes Using Chitin Nanocrystals. ACS Sustainable Chemistry Engineering. 2024, 12, 15980–15990.

[9] Calvo V., Martínez-Barón C., Fuentes L., Maser W.K., Benito A.M., González-Domínguez J.M. Nanocellulose: The Ultimate Green Aqueous Dispersant for Nanomaterials. Polymers. 2024, 16, 1664.

[10] Martínez-Barón C., Calvo V., Hernández-Ferrer J., Villacampa B., Ansón-Casaos A., González-Domínguez J.M., Maser W.K., Benito A.M. Towards Sustainable TiO2 Photoelectrodes Based on Cellulose Nanocrystals as a Processing Adjuvant. RSC Sustainability. 2024, 2, 2015–2025.

[11] Martínez-Barón C., Calvo V., Álvarez-Sánchez M.A., Maser W.K., Benito A.M., Ansón-Casaos A., González-Domínguez J.M. Carbon Nanotube Film Electrodes Enabled by Nanostructured Biopolymers through Aqueous Processing. Physical Chemistry Chemical Physics. 2025, 27, 16756-16767.

[12] Baquero-Aznar V., Calvo V., González-Domínguez J.M., Maser W.K., Benito A.M., Salvador M.L., González-Buesa J. Novel Egg White Protein–Chitin Nanocrystal Biocomposite Films with Enhanced Functional Properties. Polymers. 2025, 17(18), 2538.

[13] Calvo V., Venegas C.J., Sierra-Rosales P., Miralles-Cuevas S., Cabrera A., Maser W.K., Benito A.M., González-Domínguez J.M. Real-Time Electrochemical Sensing of a Wastewater Contaminant Using Green Nanomaterials: From Lab Optimization to Pilot-Scale Validation. Submitted. 2025.

[14] Güemes L., Calvo V. et al. Bottom-up Assembly of Cellulose Nanocrystals as a Green entry to Structured Functional Carbons. Submitted. 2025.

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