

Sustainable Nanobiopesticide Formulation via Chitosan-Coated Niosomal Encapsulation of *Bacillus Subtilis* Lipopeptides

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Abstract

The imperative to feed a growing global population has intensified reliance on synthetic agrochemicals, precipitating severe soil degradation and pest resistance. Nanobiopesticides represent a paradigm shift, utilizing nanotechnology to enhance the efficacy of biological control agents. This review critically examines the development of a specific nanocarrier system: chitosan-coated niosomes loaded with *Bacillus subtilis* lipopeptides (surfactin, iturin, and fengycin). It bridges the gap between microbiological efficacy and colloidal chemistry.

Studies were selected based on relevance to niosome formulation, chitosan surface modification, and lipopeptide bioactivity. The review identifies that while *Bacillus* lipopeptides are potent, they suffer from environmental instability. Niosomes offer a cost-effective, chemically stable alternative to liposomes for encapsulation. Surface engineering with chitosan significantly enhances mucoadhesion, UV protection, and enables pH-responsive release. The integration of stimuli-responsive mechanisms (enzyme-triggered release) and AI-driven precision agriculture utilizing these nanocarriers constitutes the next frontier in sustainable farming.

Keywords: Nanobiopesticides, *Bacillus subtilis*, Lipopeptides, Chitosan, Niosomes, Sustainable Agriculture, Smart Delivery Systems.

Introduction

Global Problem Statement

The global agricultural sector faces a paradoxical challenge: boosting food production by 70% by 2050 while mitigating the catastrophic environmental footprint of conventional farming.¹ Synthetic pesticides, characterized by low efficiency (<0.1% reaches the target), have contaminated water tables and destabilized soil microbiomes.² The indiscriminate use of these chemicals has accelerated the evolution of resistant pest populations, necessitating higher dosages and creating a vicious cycle of chemical dependency.⁴

Importance of the Topic

Biopesticides derived from *Bacillus subtilis* offer a sustainable alternative. This bacterium produces cyclic lipopeptides (CLPs)—surfactin, iturin, and fengycin—which exhibit broad-spectrum antimicrobial activity and induce systemic resistance in plants.⁴ However, their application is hindered by inherent

limitations: sensitivity to UV radiation, poor solubility, and rapid degradation in field conditions.⁵

Why This Review is Needed

Current literature often addresses *Bacillus* metabolites and nanotechnology in isolation. There is a lack of comprehensive reviews integrating the biochemical potential of CLPs with the engineering principles of chitosan-coated niosomes. This review synthesizes data on formulation parameters, release kinetics, and techno-economic feasibility to propose a viable path for commercialization.

Objectives

1. To evaluate the physicochemical advantages of niosomes over liposomes for agricultural applications.
2. To analyze the mechanism and benefits of chitosan coating for controlled release and leaf retention.
3. To assess the regulatory landscape and economic viability of this nanobiopesticide.

Core Review Sections

Conceptual Background

Bacillus subtilis Lipopeptides

The bioactive core of the proposed formulation consists of Cyclic Lipopeptides (CLPs). These amphiphilic molecules feature a cyclic peptide head linked to a fatty acid tail.⁷

- **Surfactin:** Potent biosurfactant 13 to 15 Carbon atoms. ; destabilizes membranes and inhibits biofilm formation.⁴
- **Iturin:** Strong antifungal agent 14 to 17 carbon atoms; forms ion-conducting pores in fungal membranes.⁴
- **Fengycin:** Targets filamentous fungi 15 to 17 carbon atoms; causes structural collapse of the lipid bilayer.⁴

Niosomes: The Vesicular Carrier

Niosomes are microscopic lamellar structures formed by the self-assembly of non-ionic surfactants (e.g., Span 60, Tween 80) and cholesterol.¹⁰ Unlike liposomes, which are made of phospholipids, niosomes are chemically stable and resistant to oxidation.¹⁰

Current Developments in Formulation

Surfactant Selection

Recent studies highlight Span 60 as the optimal surfactant due to its high phase transition temperature and appropriate Hydrophilic-Lipophilic Balance (HLB), which favors the formation of stable, leak-resistant vesicles. The inclusion of cholesterol at a 1:1 molar ratio is critical for cementing the bilayer and preventing leakage.

Chitosan Surface Coating

Chitosan, a cationic polysaccharide, interacts electrostatically with the negatively charged niosome surface.¹² This "core-shell" structure provides:

- **Zeta Potential Reversal:** Shifts surface charge from -40 mV to +30 mV, enhancing stability.¹²
- **Mucoadhesion:** The positive charge facilitates binding to negatively charged plant leaves and pest membranes.¹⁵
- **Size Increase:** Coating typically increases particle size from approximately 150 nm to 250–300 nm,

improving payload capacity.¹⁷

Table 1: Key Formulation Parameters from Recent Studies

Component	Optimal Parameter	Function	Reference
Surfactant	Span 60 (HLB 4.7)	Forms stable bilayer; high entrapment efficiency.	19
Stabilizer	Cholesterol (1:1 ratio)	Prevents leakage; rigidifies membrane.	20
Coating	Chitosan (0.1% - 0.5% w/v)	Provides mucoadhesion; controls release.	21
Solvent	Acetic Acid (pH 4-5)	Solubilizes chitosan for coating.	12
Method	Thin Film Hydration	Simple; reproducible; high yield.	22

Comparative Analysis

Niosomes vs. Liposomes

While liposomes are the gold standard in medicine, they are ill-suited for agriculture due to cost and instability.

Feature	Liposomes (Phospholipids)	Niosomes (Non-ionic Surfactants)	Agricultural Implication
Cost	High (expensive precursors)	Low (cheap commodity chemicals)	Niosomes are economically viable for large-scale use. ¹⁰
Stability	Low (oxidation/hydrolysis)	High (chemically stable)	Niosomes have longer shelf-life without cold chain. ²⁴
Storage	Requires 4°C or freezing	Stable at room temp	Niosomes reduce logistical burdens. ¹¹
Toxicity	Biocompatible	Biocompatible/Biodegradable	Both are safe, but niosomes degrade slower in soil. ²⁶

Encapsulated vs. Free Lipopeptides

Free lipopeptides degrade rapidly under UV light and high temperatures. Encapsulation in chitosan-coated niosomes extends their half-life significantly. Studies show that encapsulated formulations retain >80% efficacy after 14 days, whereas free lipopeptides lose activity within 48 hours.²⁷

Applications

Disease Control

The formulation has demonstrated high efficacy against necrotrophic fungi. The dual mechanism involves the direct pore-forming activity of the lipopeptides and the disruption of fungal cell walls by the chitosan shell.²⁹

- *Case Study:* Treatment of *Fusarium solani* in tomato plants showed 100% reduction in disease symptoms using *Bacillus* lipopeptides, comparable to commercial fungicides.²⁹
- *Case Study:* Chitosan-coated systems showed enhanced efficacy against *Botrytis cinerea* (grey mould) on strawberries due to improved retention on the fruit surface.³⁰

Smart Delivery Systems

Recent advances (2024-2025) focus on stimuli-responsive release.

- **Enzyme-Triggered:** Niosomes can be engineered to degrade only in the presence of specific fungal enzymes (e.g., pectinases), releasing the payload exactly when infection occurs.¹
- **pH-Triggered:** The chitosan shell swells in acidic environments (characteristic of some fungal infections), triggering release.¹²

Limitations and Challenges

Regulatory Hurdles

The regulatory framework for nanopesticides is fragmented.

- **EU:** Stringent regulations (REACH, Regulation 2018/1881) require specific risk assessments for "nanoforms," creating high barriers to entry.³
- **USA:** Regulated under FIFRA; while there is no specific "nano-act," the EPA requires extensive data on exposure and safety.³

Scalability

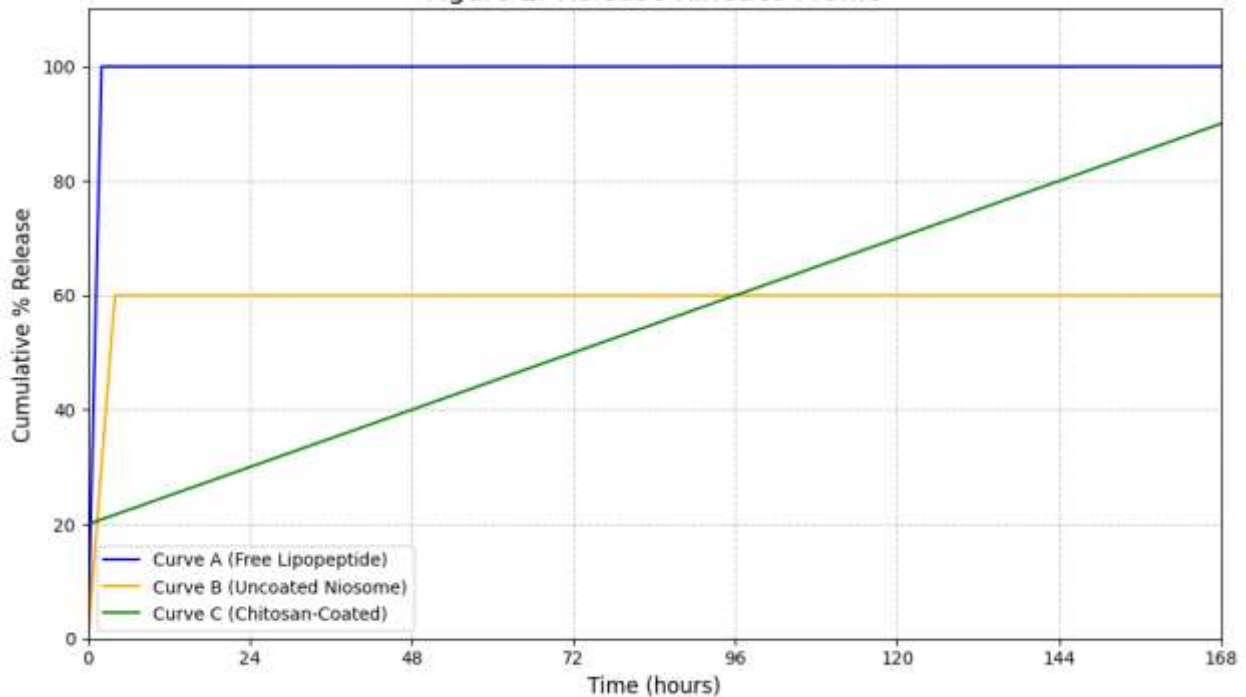
Transitioning from laboratory methods (Thin Film Hydration) to industrial production (Microfluidics or High-Pressure Homogenization) remains a challenge. While microfluidics offers precise control, high throughput production at low cost is difficult to achieve.²²

Tables & Figures



Figure 1: Schematic representation of the Chitosan-coated niosomal system. The design features a hydrophilic core loaded with Surfactin/iturin and a hydrophobic bilayer entrapping Fengycin. The external Chitosan coating provided zeta-potential reversal (cationic charge) to facilitate mucoadhesion to negatively charged plant leaf surfaces.

Figure 2: Release Kinetics Profile



- **Curve A (Free Lipopeptide):** Shows 100% cumulative release within the first 2 hours, representing a rapid burst.
- **Curve B (Uncoated Niosome):** Depicts a release of approximately 60% within 4 hours, after which the release plateaus.
- **Curve C (Chitosan-Coated):** Starts with a 20% initial burst (shock dose) and then demonstrates a sustained, approximately zero-order (linear) release over 7 days, reaching around 90% by the end of the period.

Future Perspectives

Integration with Precision Agriculture .The next generation of nanobiopesticides will integrate with AI and drone technology. Niosomes could be loaded with tracers that allow drones to detect coverage and pest pressure in real-time."All-Organic" SystemsFuture research should focus on replacing synthetic surfactants (Spans/Tweens) with fully bio-based biosurfactants (e.g., sophorolipids) to create a 100% renewable "all-organic" delivery system, further minimizing environmental impact. Long-term Soil ToxicityThere is a gap in longitudinal studies regarding the fate of niosomes in soil. While components are biodegradable, the long-term impact of chronic nanoscale exposure on soil microbiomes needs rigorous assessment.

Conclusion

The chitosan-coated niosomal encapsulation of *Bacillus subtilis* lipopeptides represents a pivotal advancement in sustainable agriculture. By addressing the instability and short shelf-life of biological agents, this technology unlocks the full potential of biopesticides. The formulation offers a synergistic effect: niosomes provide a stable, cost-effective reservoir, while chitosan ensures targeted delivery and environmental protection. Overcoming the regulatory bottlenecks and scaling up production technologies will be the final steps in transitioning this innovation from the laboratory to the field, offering a potent tool for global food security.

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