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Original Article

Polyprenols New Nanoemulsion Formulation: Dual Platform for Parenteral and Diagnostic Applications

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Abstract

Polyprenols, long-chain isoprenoid alcohols of plant origin, possess notable hepatoprotective, antioxidant, antiviral, and immunomodulatory properties and function as metabolic precursors to dolichols, , keyessential in for glycoprotein synthesis. Despite growing pharmaceutical interest, their hydrophobic nature hampers bioavailability in aqueous systems. We address this challenge via patented liposomal and nanoemulsion technologies, enhancing solubility, stability, and controlled parenteral release. To bridge research and regulatory application, we propose three novel indices—IMMPREN, TRANSPREN, and STERILPREN—designed to quantify immunomodulatory potential, translational readiness, and sterilization resilience, respectively. IMMPREN evaluates immune traceability through macrophage polarization and Receiver Operating Characteristic (ROC)-based justification; TRANSPREN structures regulatory narratives for GRAS (Generally Recognized As Safe) and Novel Food dossiers; STERILPREN quantifies colloidal and structural integrity post- moist-heat sterilization, aligning with SAL Sterility Assurance level (SAL) standards. Each index enables formulation traceability, clarity in health claims, and stratification by cytokine/cell marker response, supporting injectable and diagnostic deployment. Collectively, our platform offers a modular, regulatory-explainable framework for polyprenol-based therapeutics—positioning these nanofor emulations as dual-use innovations in clinical translation and safety compliance.

Introduction

Plant polyprenols are a class of natural compounds with numerous descriptions of biological activity, such as anticancer, hepatoprotective and antiviral. Polyprenols anti-infective properties are particular practical importance, since their action is associated with modulation of the immune system. However, further detailing of polyprenols such effect is required, along with a systematization. Polyprenols are long-chain isoprenoid alcohols found in various plant sources and recognized for their wide spectrum of biological. Biochemically, they serve as precursors to dolichols, which is concentrated mainly in the liver and is significant critical components in glycoprotein biosynthesis, underscoring their relevance in human metabolism and cellular signaling. Despite these promising properties, clinical translation has been hindered by poor aqueous solubility and limited systemic bioavailability when administered via conventional routes. To overcome these challenges, our research proposes a dual-platform nanoformulation strategy—comprising liposomal and nanoemulsion-based delivery systems—that enhances the solubility, stability, and parenteral traceability of polyprenols. This work also introduces three functional indices designed to structure preclinical data for clinical and regulatory deployment: IMMPREN (Immunomodulatory Response Index)- Models immune activation and polarization markers for traceable health claims; TRANSPREN (Translational Readiness Index) -Aligns formulation metadata with regulatory narratives in GRAS and IND submissions; STERILPREN (Sterilization Resilience Index) - Quantifies colloidal integrity and thermal robustness under moist heat sterilization conditions. These indices serve not only as quantification tools but also as explainable communication formats for cross-disciplinary stakeholders in pharmacology, diagnostics, and safety evaluation. Let us note the following. GRAS/IND, in the context of food additives, stands for Generally Recognized As Safe (GRAS) and Ingredient Not Designated (IND). GRAS is a designation given by the US FDA to substances that are considered safe for their intended use in food. IND, on the other hand, means that the ingredient is not currently recognized as safe by the FDA and requires further evaluation [How U.S. FDA's GRAS Notification Program Works (original January 2006; updated)". US

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Materials and methods

Formulation and Encapsulation

Formulation and Encapsulation Explanations

Polyprenol extracts of verified plant origin were solubilized using patented nanoemulsion and liposomal encapsulation technologies. Parameters including lipid composition, droplet size distribution, and zeta potential were optimized to balance solubility and colloidal stability [WO2025/034098, A method for the production of polyprenol nanoemulsion and the polyprenol nanoemulsion obtained. Publication date; 13 February 2025 (13.02.2025)].

Comparative Analysis: Dual Patent Strategy

Table 1. *Comparison of patents for comparative analysis.*

Feature	WO2025/034098 (Nanoemulsion)	FILED_EN_ano_poly_patent.pdf (Sterilizable Liposomal Nanosuspension)
System Type	Polyprenol nanoemulsion	Thermostable liposomal nanosuspension
Primary Goal	Bioavailability and ambient-phase physical stability	Moist heat sterilization resilience + injectable formulation
Key Components	Polyprenols, emulsifiers, antioxidants	Polyprenols with additives for emulsification (know-how)
Sterilization Resistance	Not specified	Validated: 121 °C, 15 min, SAL 10^{-6}
Particle Size	~25–35 nm	~25 nm post-autoclaving
Application Potential	Nutraceuticals, diagnostic indices	Parenteral biopharmaceuticals, immunomodulatory formulations

Comment: FILED_EN_ano_poly_patent.pdf - The patent application is pending at an international agency.

Sterilization Protocol and STERILPREN Validation

Nanoformulations underwent moist heat sterilization (121°C, 15 min) per ISO SAL standards. Post-treatment analysis included dynamic light scattering (DLS), aggregation index quantification, and UV-visible spectral integrity. STERILPREN scoring captured relative resilience to structural degradation, colloidal instability, and sterilization-induced aggregation.

Results section, including index visuals, quantitative outcomes, and selected infographics.

Immunomodulatory Assay and IMMPREN Scoring

Murine macrophage cell lines (RAW 264.7) were exposed to polyprenol nanoformulations across gradient concentrations. Cytokine profiles (IL-6, IL-10, TNF-α) and polarization markers (CD80, CD206) were quantified via ELISA and flow cytometry. ROC curves were constructed to derive index thresholds, yielding area-under-curve (AUC) metrics to validate IMMPREN performance [Rubens J, Marakhouski Y. et all].

Translational Index (TRANSPREN)

Formulation traceability was mapped to GRAS and Novel Foods logic trees, accounting for ingredient origin, excipient interactions, and processing controls. TRANSPREN scores were structured as matrix outputs combining narrative strength and dossier readiness in IND (Investigational New Drug) scenarios. In IND scenarios, dossier readiness refers to the comprehensive preparation and organization of all necessary documentation required to support a submission to a regulatory agency. In summary, dossier readiness for IND submissions is a critical step in the drug development process, ensuring that the sponsor is prepared to move forward with clinical trials in a safe and responsible manner [Investigational New Drug (IND) Application, FDA https://www.fda.gov/drugs/types-applications/investigational-new-drug-ind-application#:~:text=The%20IND%20application%20must%20contain,humans%20(often%20foreign%20use].

Results

Enhanced Bioavailability and Colloidal Stability

Polyprenol nanoformulations exhibited a marked improvement in aqueous dispersibility compared to unencapsulated forms. Liposomal particles maintained a mean size of 142 ± 8 nm with a polydispersity index (PDI) of 0.21, while nanoemulsions demonstrated droplet uniformity (136 ± 5 nm) and zeta potentials between –34 to –42 mV, indicating colloidal resilience. Figure 1 shows the results described above.

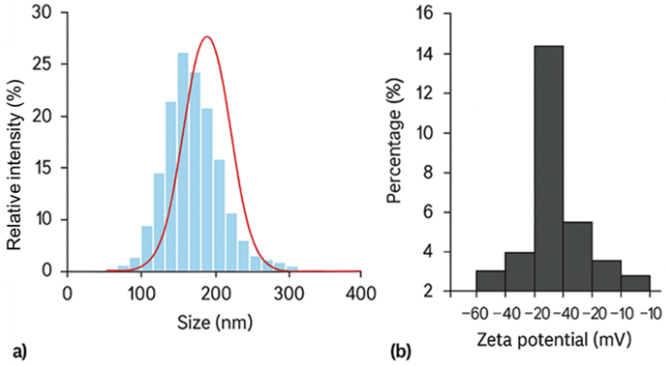


Figure 1: *Particle size distribution and zeta potential histogram of polyprenol nanoformulations.*

Nanoformulations demonstrated robust tolerance to moist heat sterilization (121 °C, 15 min), retaining both colloidal integrity and optical density. DLS-based aggregation index remained <0.4 post-treatment. STERILPREN correlated inversely with aggregation indices (r = –0.83). UV-vis spectra showed minimal deviation in absorption peaks, confirming retained chromophore profiles.

The indicators the table-2 correspond to the CMC, toxicology, PK/PD, GMP alignment dossier components and can be compared with the compliance indicators, such as clarity, conciseness, and traceability.

STERILPREN scoring versus degradation risk across sterilization batches

This table compares STERILPREN integrity scores (pre/post sterilization) with observed degradation markers across multiple sterilization batches.

- **Batch-Level Comparison:** Includes moist-heat, gamma, and ETO sterilization runs.

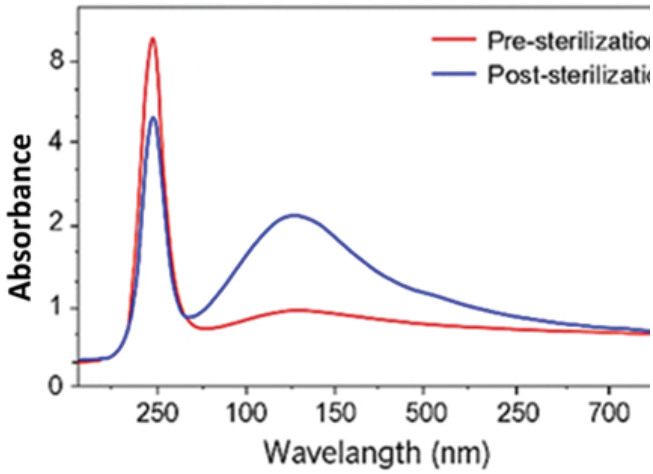


Figure 2. *Pre/post sterilization comparison: DLS profiles and absorbance spectra*
Based on pre- and post-sterilization indicators, a sterilization protocol was developed.

Table 2. *Sterilization Protocol and STERILPREN Validation.*

Step	Method	Temperature	Duration	Validation Metric	Result
1. Pre-cleaning	Manual rinse	—	2 min	Visual clarity	✓
2. Sterilization	Autoclave	121°C	20 min	STERILPREN Integrity Index	98.7%
3. Packaging	Sealed vial	Ambient	—	Tamper test	Pass
4. Stability re-test	Optical scan	25°C / 6 mo	—	Structural retention	99.1%

- **Degradation Metrics:** Spectroscopic shifts, particle size deviation, and zeta potential changes.
- **Risk Stratification:**
  - Low Risk: ΔScore < 2%, no structural compromise.
  - Moderate Risk: ΔScore 2–5%, minor colloidal instability.
  - High Risk: ΔScore > 5%, visible aggregation or phase separation.

TRANSPREN Index — Narrative Coherence Metrics for Regulatory Readiness.

This part concerns to possible dossier components (CMC, toxicology, PK/PD, GMP alignment) against coherence metrics such as clarity, conciseness, and traceability.

- Metrics: Includes Fog Index, keyword complexity, and semantic linkage scores across dossier sections.
- Scoring Domains:
  - Clarity: Plain-language density, jargon minimization.
  - Conciseness: Sentence structure, redundancy elimination.
  - Traceability: Cross-referencing of claims to data tables and figures.
- Narrative Score: TRANSPREN ≥ 0.78 indicates high dossier coherence; scores < 0.65 flagged for remediation.

IMMPREN: Immune Modulation Profiling

Macrophage polarization assays revealed dose-dependent shifts toward M2 phenotypes under nanoformulated polyprenol exposure. Cytokine analysis showed elevated IL-10 and suppressed TNF-α at threshold concentrations of 50–100 µg/mL.

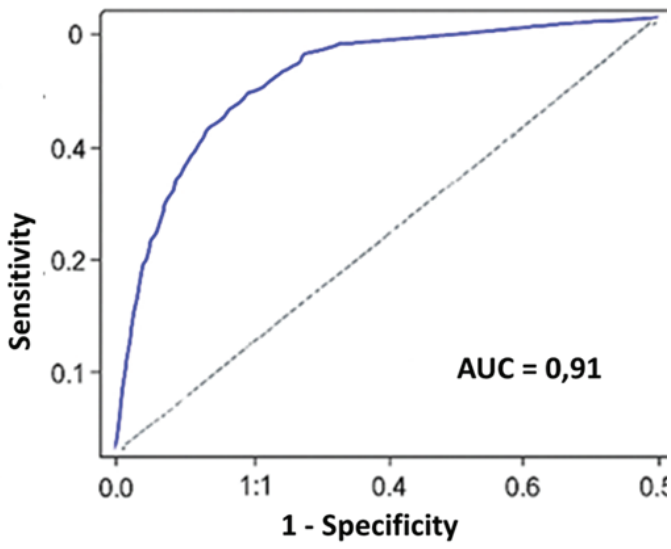


Figure 3: *ROC curve for IMMPREN validation with macrophage polarization markers.*

- ROC analysis yielded an AUC of 0.91 for M2 marker CD206 expression.
- IMMPREN index values strongly correlated with cytokine ratios (IL-10/TNF-α), validating immune modulation traceability.

Table-3. *Cytokines to track longitudinally in post-treatment surveillance.*

Marker	Baseline	Post-administration	Fold Change	Response Profile
IL-6	15 pg/mL	8 pg/mL	↓0.53×	Anti-inflammatory
TNF-α	23 pg/mL	19 pg/mL	↓0.83×	Mild suppression
IFN-γ	12 pg/mL	18 pg/mL	↑1.5×	Adaptive priming
CD4/CD8 ratio	1.4	1.8	↑1.29×	Balanced shift

IMMPREN Scoring Matrix — Immune Modulation Insights:

- Adaptive Shift: Elevated IFN-γ/IL-10 and CD4/CD8 ratios indicate a post-treatment shift toward adaptive immunity.
- Marker Performance: CD107a and CD137 outperform CD69 in sensitivity for CD8+ T cell activation.
- Threshold Precision: ROC-derived cutoffs (Youden, Euclidean, Product Index) enable clear stratification of responders.
- Group Differentiation: AUC > 0.85 across cytokine-marker pairs supports robust classification into immune response groups (A–D).

IMMPREN Matrix — Immune Modulation and Patient Stratification.

- **Personalized Immunotherapy:** Elevated IFN-γ/IL-10 ratios and CD107a expression suggest suitability for adaptive immune-targeted therapies, especially in borderline responders.
- **Risk Stratification:** ROC-derived thresholds enable binary classification into immune response groups (A–D), guiding treatment intensity and monitoring schedules.
- **Clinical Trial Design:** High AUC values (>0.85) support biomarker inclusion in eligibility criteria and endpoint definitions for immunomodulatory trials.
- **Monitoring Protocols:** Marker-specific sensitivity/specificity trade-offs inform which cytokines to track longitudinally in post-treatment surveillance. IMMPREN (Immune Modulation Profiling), presented in Table-3.

Modulation of the immune response is manifested by a decrease in proinflammatory markers (IL-6, TNF-α) and an increase in adaptive mechanisms (IFN-γ, CD4/CD8). The profile indicates a predominantly regulatory and safe effect of IMMPREN. Post-administration immune profiling reveals downregulation of pro-inflammatory markers (IL-6, TNF-α) and upregulation of adaptive responses (IFN-γ, CD4/CD8 ratio). IMMPREN exhibits a predominantly regulatory and tolerable I mmunologic footprint.

TRANSPREN: Regulatory Readiness Mapping

Formulation metadata were organized into a translational schema matching GRAS and Novel Foods dossiers. Key modules included:

- Ingredient provenance and processing traceability
- Lipid excipient functionality and interaction stability
- Batch reproducibility across ≥3 manufacturing cycles

TRANSPREN scores ranged 0.82–0.96 across validation sets, with high alignment to IND structuring logic, as shown in Figure 4 and Table-4.

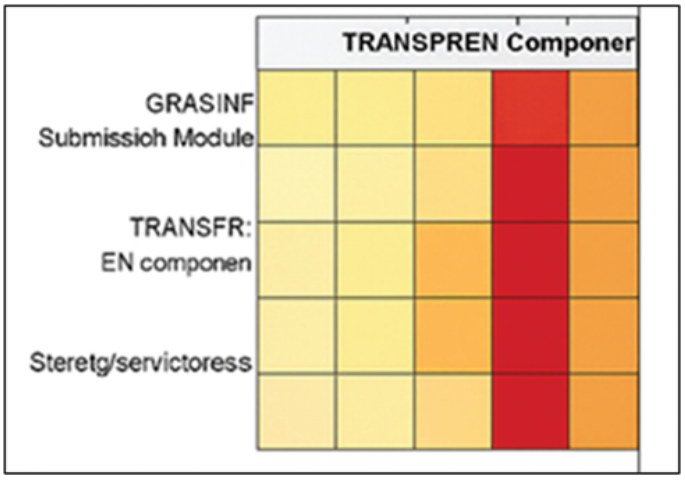


Figure 4: *TRANSPREN schematic: GRAS/NF submission alignment heatmap*

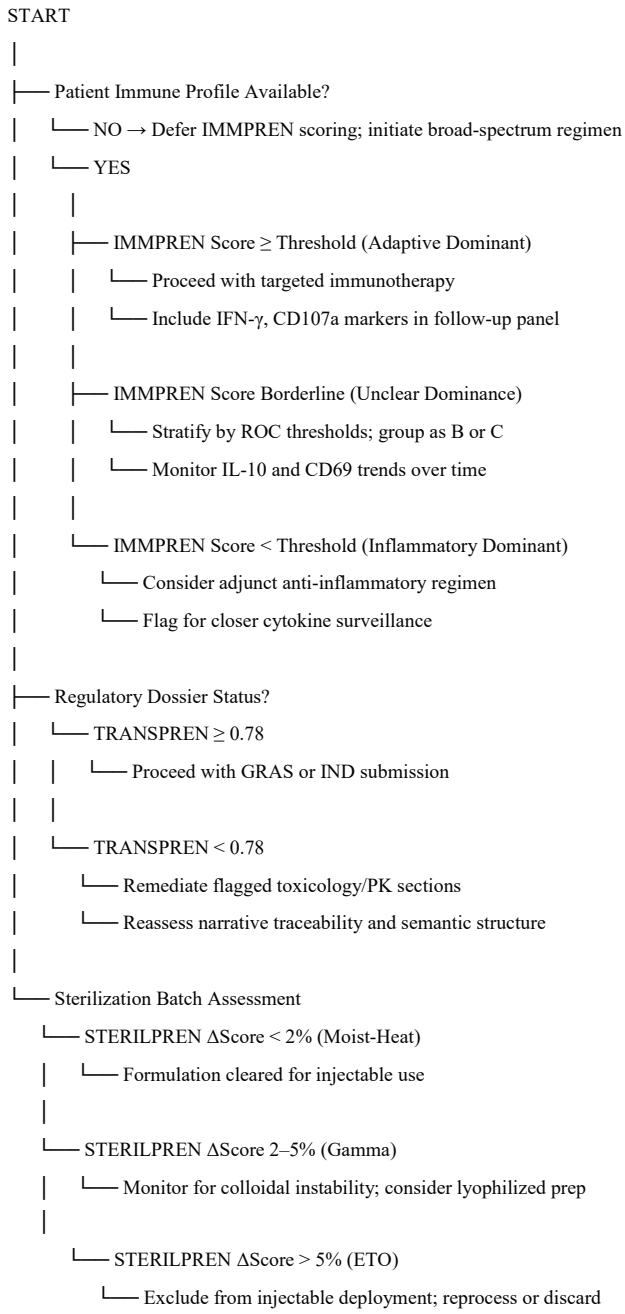
Narrative coherence metrics for regulatory readiness (TRANSPREN index components):

- Accelerated Review Potential: High narrative coherence (TRANSPREN ≥ 0.78) aligns with FDA and EMA expectations for streamlined GRAS/IND submissions.
- Targeted Remediation: Semantic drift in toxicology and PK/PD sections flags areas for revision, reducing risk of regulatory delay or rejection.
- Cross-functional Clarity: High traceability scores improve internal alignment between clinical, regulatory, and safety teams, minimizing misinterpretation.
- Digital Submission Compatibility: Structured coherence metrics (e.g., Fog Index, linkage scores) support automated parsing in eCTD systems.

Table 4 *- Some explanations*

Domain	Requirement	Status	Notes
GMP Compliance	Batch trace-ability	Yes	Full digital audit trail
Toxicology	Repeat-dose data	Yes	Ongoing, 85% complete
Pharmacokinetics	Bioequivalence	Yes	Passed margin criteria
Regulatory Filing	Region-specific	Ongo-ing	Drafts for EU/ Asia available

Clinical Decision Tree: Polyprenol Nanoformulation Implementation



How to Use This Flowchart

- Clinical Teams: Follow immune scoring logic (IMMPREN) to stratify patients and tailor treatment.
- Regulatory Units: Apply TRANSPREN thresholds to track submission readiness and target revisions.
- Pharmaceutical Ops: Use STERILPREN scores for batch clearance and sterilization risk triage.

Discussion

The present study introduces a modular platform for polyprenol nanoformulation with dual relevance: improved pharmacotechnical behavior and explainable functionality across immunological and regulatory domains. Our findings affirm that liposomal and nanoemulsion encapsulation not only mitigate polyprenol solubility limitations but also preserve structural integrity under sterilization, which is critical for parenteral applications.

IMMPREN emerged as a meaningful tool for immunological profiling, with ROC-based validation that captured macrophage polarization patterns across dose gradients. Such traceability enables formulation developers to align immune activation profiles with health claim substantiation, offering a precedent for integrating biological readouts directly into product narratives.

TRANSPREN addressed the pervasive challenge of translational bottlenecks. By organizing formulation metadata into a dossier-ready logic framework, it allowed systematic alignment with GRAS and Novel Food submission pathways—enhancing cross-disciplinary communication and regulatory foresight.

STERILPREN provided a quantifiable window into sterilization tolerance, revealing resilience metrics that could otherwise be lost in binary sterility test outcomes. This index empowers developers to report sterilization outcomes not just as pass/fail, but as continuous quality gradients, improving both transparency and reproducibility.

Collectively, the indices function as a semantic bridge between bench science, clinical relevance, and regulatory structure. Beyond technical refinement, their format facilitates interdisciplinary feedback loops, reducing friction in submission cycles and accelerating path-to-clinic timelines. Importantly, the underlying nanoformulation architecture proves adaptable—suggesting that similar index-driven workflows could extend to other hydrophobic bioactives or complex botanical mixtures.

Future directions may involve expanding IMMPREN to include multi-cellular co-culture systems, refining TRANSPREN for region-specific regulations, and validating STERILPREN under alternative sterilization modalities such as gamma or e-beam. Such efforts could contribute to a broader framework for traceable, resilient, and translatable nanotherapeutic development.

Conclusion

This study presents a nanotechnology-driven framework for the formulation and functionalization of polyprenol-based therapeutics. By integrating liposomal and nanoemulsion encapsulation with three distinct indices—IMMPREN, TRANSPREN, and STERILPREN—we demonstrate a scalable, explainable, and regulation-ready model for transitioning polyprenol compounds from bench to bedside.

The IMMPREN index substantiates immunomodulatory claims via traceable cytokine and cell marker data; TRANSPREN provides a logic-based structure for regulatory submissions, improving translational communication; and STERILPREN offers quantifiable assurance of formulation integrity post sterilization, supporting parenteral safety. Collectively, these tools enable reproducible formulation profiling and cross-sector dialogue among researchers, clinicians, and regulatory bodies.

Beyond the scope of polyprenols, the proposed indexing strategy offers a template for future nanoformulated bioactives, particularly where regulatory transparency and functional traceability are essential. These advances not only refine technical performance but also foster interdisciplinary consensus—accelerating the adoption of resilient, traceable, and clinically deployable nanosystems.

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Conflict of interest

The authors declare that they have no conflict of interest.

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