Available online on 15.11.2025 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Review Article

Adaptive Nanocarriers: A New Era in Transdermal Delivery with Transferosomes

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Article Info:

Article History:

Received 13 Aug 2025
Reviewed 07 Oct 2025
Accepted 30 Oct 2025
Published 15 Nov 2025

Cite this article as:

Shadab A, Ansari AF, Adaptive Nanocarriers: A New Era in Transdermal Delivery with Transferosomes, Journal of Drug Delivery and Therapeutics. 2025; 15(11):72-84 *DOI: http://dx.doi.org/10.22270/jddt.v15i11.7426*

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Abstract

Transferosomes represent a transformative class of ultra-deformable, elastic nanocarriers that have redefined the potential of transdermal drug delivery. Unlike conventional liposomes, which are largely restricted to topical applications, transferosomes possess unique membrane architectures incorporating edge activators such as surfactants or bile salts that confer exceptional flexibility and enable them to navigate the skin's impermeable barrier via intracellular and transappendageal routes. This self-optimizing capability allows for non-invasive systemic delivers a very wide range of therapeutics, including macromolecules, vaccines, and hydrophobic drugs, with enhanced bioavailability and minimal irritation. Recent advances in microfluidic synthesis, quality-by-design (QbD) frameworks, and intelligent process controls have accelerated the development of robust, scalable manufacturing platforms for transferosomes. Meanwhile, the integration of machine learning and molecular dynamics simulations offers unprecedented predictive power in formulation design, optimizing traits such as vesicle size, encapsulation efficiency, and membrane elasticity. Regulatory pathways are also evolving, with growing emphasis on critical quality attributes like deformability index, stability under stress, and in vitro-in vivo correlation. This review comprehensively examines the biophysical principles governing transferosome behavior, state-of-the-art production techniques, and cutting-edge characterization tools essential for clinical translation. It also highlights emerging applications in personalized medicine, combinatorial therapy, and targeted dermatological treatments. As the pharmaceutical industry shifts toward patient-centric, non-invasive delivery solutions, transferosomes stand at the forefront bridging scientific innovation with tangible therapeutic impact and paving the way for a new era in transdermal nanomedicine.

Keywords: Transferosome, Transdermal Drug Delivery, Edge Activators, Skin Penetration Mechanism, Quality by Design (QbD), Nanocarrier Characterization, Scalability.

1. INTRODUCTION

Transdermal drug delivery has emerged as a critical frontier in pharmaceutical sciences, offering an attractive alternative to conventional administration routes by bypassing gastrointestinal degradation and first-pass metabolism while enabling sustained release profiles[1]. However, the stratum corneum the outermost layer of the epidermis—has historically presented a formidable barrier, limiting this route to small, lipophilic molecules with minimal molecular weights. The advent of vesicular nanocarriers marked a significant breakthrough, with liposomes initially showing promise for topical applications[2 3]. Yet, their inability to penetrate intact skin beyond superficial layers constrained therapeutic potential. The paradigm shifted with the introduction of transferosomes in the early 1990s ultradeformable, elastic vesicles engineered to overcome cutaneous barriers through intentional membrane destabilization. These second-generation liposomal variants incorporate "edge activators" that confer exceptional flexibility, enabling them to squeeze through intercellular pores measuring less than one-tenth of their diameter[1 4]. This unique property facilitates not only localized deep tissue delivery but also systemic circulation, opening avenues for macromolecular therapeutics previously deemed unsuitable for transdermal administration[3].

The clinical relevance of transferosomes continues to expand, with demonstrated success in delivering insulin, vaccines, analgesics, and biologics. Recent advances in nanofabrication, quality-by-design (QbD) approaches, and intelligent characterization tools have accelerated their transition from experimental systems to viable commercial products. Furthermore, the integration of computational modeling and machine learning has begun to unravel the complex structure-function relationships governing their behavior, paving the way for rational design and optimization[4 5].

This review comprehensively examines the transformative potential of transferosomes in bridging the gap between laboratory innovation and clinical translation. We delve into their structural biophysics,

mechanisms of skin penetration, production methodologies, characterization benchmarks, and regulatory pathways. By synthesizing recent breakthroughs and identifying persistent challenges, we aim to provide a roadmap for the continued evolution of transferosome-based therapeutics in an increasingly personalized medical landscape[5 6].

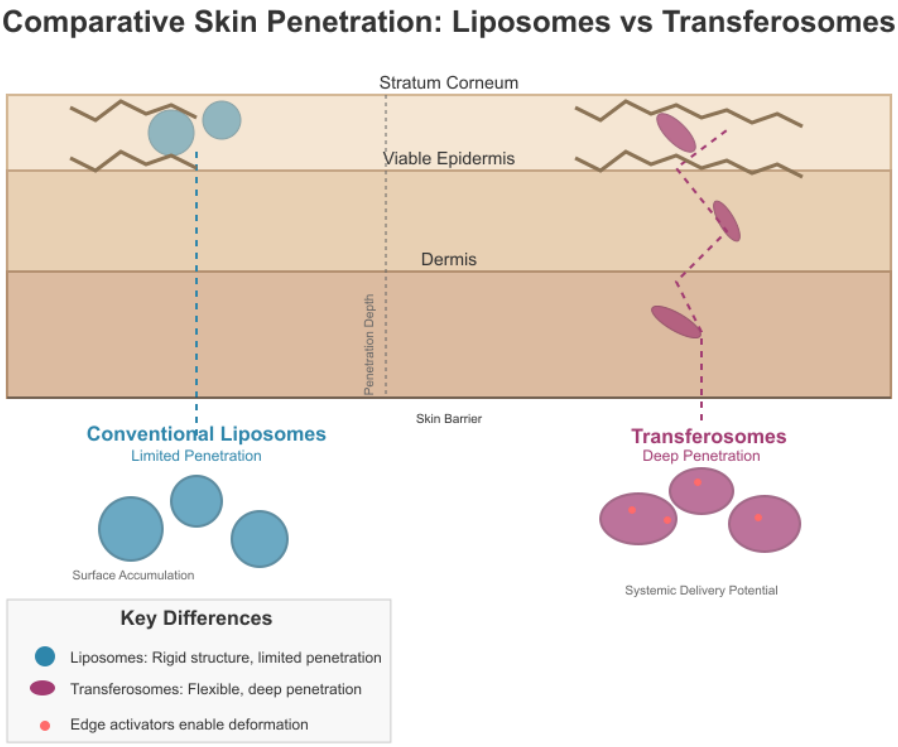

Figure 1: Schematic comparison of conventional liposomes vs transferosomes penetrating skin layers [4 16 54].

2. TRANSFEROSOME STRUCTURE AND COMPOSITION

2.1. Fundamental Architecture- Transferosomes possess a bilayer structure similar to conventional liposomes but with critical modifications that enable their unique deformability. The primary components include:

· Phospholipids: Phosphatidylcholine (PC) remains the most commonly used structural lipid, forming the backbone of the bilayer. Unlike rigid liposomal membranes, transferosomal bilayers exhibit dynamic fluidity due to the incorporation of membrane destabilizers.

· Edge Activators: These surfactant molecules (e.g., sodium cholate, Tween 80, Span 80, deoxycholate) are integral to transferosome function. By localizing in high-density regions within the bilayer, they create "weak spots" that allow membrane deformation under stress without compromising vesicle integrity.

· Aqueous Core: Hydrophilic drugs are encapsulated within the internal aqueous compartment, while amphiphilic and lipophilic compounds partition into the flexible bilayer[11 12 14 52].

2.2 Composition-Property Relationships- The mechanical properties of transferosomes are directly governed by their compositional ratios. The critical packing parameter (CPP) is significantly influenced by the phospholipid-to-edge-activator ratio, typically ranging from 4:1 to 8:1. Higher edge activator concentrations increase deformability but may compromise encapsulation efficiency and membrane stability[14 53]. Recent studies have demonstrated that hybrid formulations incorporating cholesterol derivatives or ceramides can optimize this balance, enhancing both elasticity and drug retention[12 97].

The surface charge of transferosomes can be modulated through the inclusion of charged lipids such as phosphatidylserine (negative) or stearylamine (positive). While neutral formulations generally exhibit superior skin compatibility, slight negative charges have been shown to improve stability through electrostatic repulsion and may facilitate interaction with hair follicles[12 14].

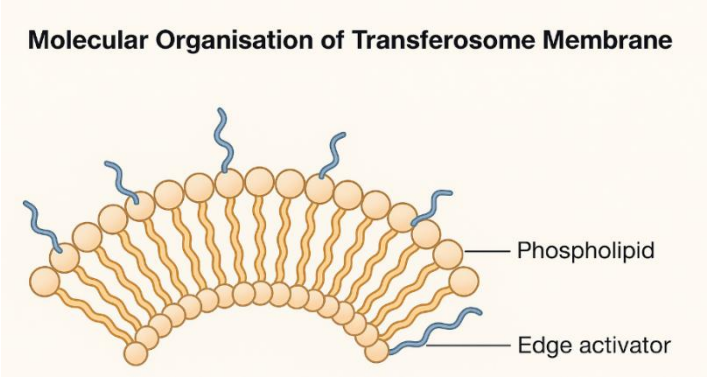

Figure 2: Molecular Organisation of transferosome membrane showing phospholipids and edge activators[32].

3. MECHANISMS OF SKIN PENETRATION: THE TRANSFEROSOME ADVANTAGE

Transferosomes employ a sophisticated multi-mechanistic approach to overcome the skin's formidable barrier properties, setting them apart from conventional vesicular systems. Their penetration capability stems from intrinsic membrane properties and responsive behavior to environmental cues.

3.1. The Deformation-Squeezing Mechanism

The cornerstone of transferosome penetration lies in their exceptional membrane deformability. While conventional liposomes maintain rigid spherical structures, transferosomes can reversibly change shape to navigate through intercellular spaces measuring only 5-30 nm—significantly smaller than their typical diameter of 100-300 nm[53 54].

Molecular Basis: · Edge Activator Function: Surfactant molecules (sodium cholate, Tween 80, etc.) create localized membrane "defects" by occupying larger interfacial areas while having single hydrophobic tails[119 120].

· Energy-Efficient Bending: The bending modulus of transferosome membranes (~10 kT) is significantly lower than conventional liposomes (~40 kT), enabling deformation with minimal energy expenditure

· Self-optimizing Geometry: Under stress, transferosomes adopt elliptical or sigmoidal shapes that minimize surface tension while maintaining structural integrity[55 56].

Penetration Dynamics:

Vesicle Deformation Process: Spherical (resting) → Elliptical (entering pore) → Sigmoidal (transiting) → Spherical (recovering)[98 99].

This shape-shifting capability allows transferosomes to squeeze through tight intercellular lipid matrices without rupture—a property quantitatively measured by deformability indices typically ranging from 15-45 for effective penetration[7 6 58].

3.2. Trans-epidermal Osmotic Gradient Drive

Transferosomes exhibit unique hydro-responsive behavior, actively moving along the skin's natural hydration gradient:

Gradient Characteristics:

· Stratum Corneum: ~15% humidity, creates "pulling" effect

· Viable Epidermis: ~50% humidity, moderate driving force

· Dermis: ~80% humidity, acts as hydration "sink"[57]

Mechanistic Steps:

. Surface Application: Transferosomes distribute across skin surface

. Water Evaporation: Creates local dehydration at application site

. Osmotic Response: Vesicles move toward higher hydration regions

. Continuous Migration: Sustained movement along hydration gradient

This active migration distinguishes transferosomes from passive diffusion systems and enables deeper penetration than size-based predictions would suggest[6 8 60].

3.3. Intercellular Lipid Reorganization

Emerging evidence suggests transferosomes can temporarily modulate skin barrier properties through lipid interactions:

Mechanistic Interactions:

· Fluidization: Edge activators can interact with intercellular ceramides and cholesterol, increasing lipid mobility

· Temporary Pore Formation: Membrane components may integrate with skin lipids, creating transient pathways

· Barrier Recovery: Unlike chemical enhancers, these interactions are reversible with minimal long-term disruption

Experimental evidence shows a 2.3-3.8 fold increase in skin permeability when using transferosomes compared to conventional liposomes, with complete barrier recovery within 24 hours[9 61 116].

3.4. Transappendageal Bypass Routes

Transferosomes utilize skin appendages as alternative penetration pathways:

Follicular Targeting:

· Size Optimization: 300-600 nm vesicles show preferential follicular accumulation

· Reservoir Function: Hair follicles act as long-term depots with sustained release

· Pilosebaceous Delivery: Direct targeting of sebaceous glands for dermatological applications

Sweat Gland Penetration:

· Conduit Function: Glandular ducts provide direct access to deeper skin layers

· Ionic Influence: Surface charge modifications can enhance glandular uptake[7 10 62 146].

Table 1: Comparative penetration efficiency of different vesicular systems

Parameter	Conventional Liposome	Transferosomes	Enhancement Factor
Stratum Corneum Penetration	Superficial (≤10 µm)	Deep (30-80 µm)	4-8x
Transdermal Flux (ug/cm^2/h)	0.5- 2.0	3.5-12.5	3-6x
Systemic delivery Efficiency	Limited	Significant	5-15x
Deformability Index	2-8	15-45	4-8x
Hydration Response	Passive	Active	N/A

3.5. Factors Influencing Penetration Efficiency

Formulation-Dependent Factors:

· Edge Activator Concentration: Optimal at 10-25% w/w of lipid content

· Membrane Fluidity: Governed by phospholipid saturation and cholesterol content

· Surface Charge: Slight negative charge (-15 to -25 mV) enhances stability without compromising penetration[51 63 147]

Application-Dependent Factors:

· Hydration Level: Moderate occlusion (30-50%) enhances osmotic drive

· Temperature: Penetration increases with skin temperature (32-37°C optimal)

· Massage: Gentle application pressure improves initial skin contact and distribution[8 10 96 118].

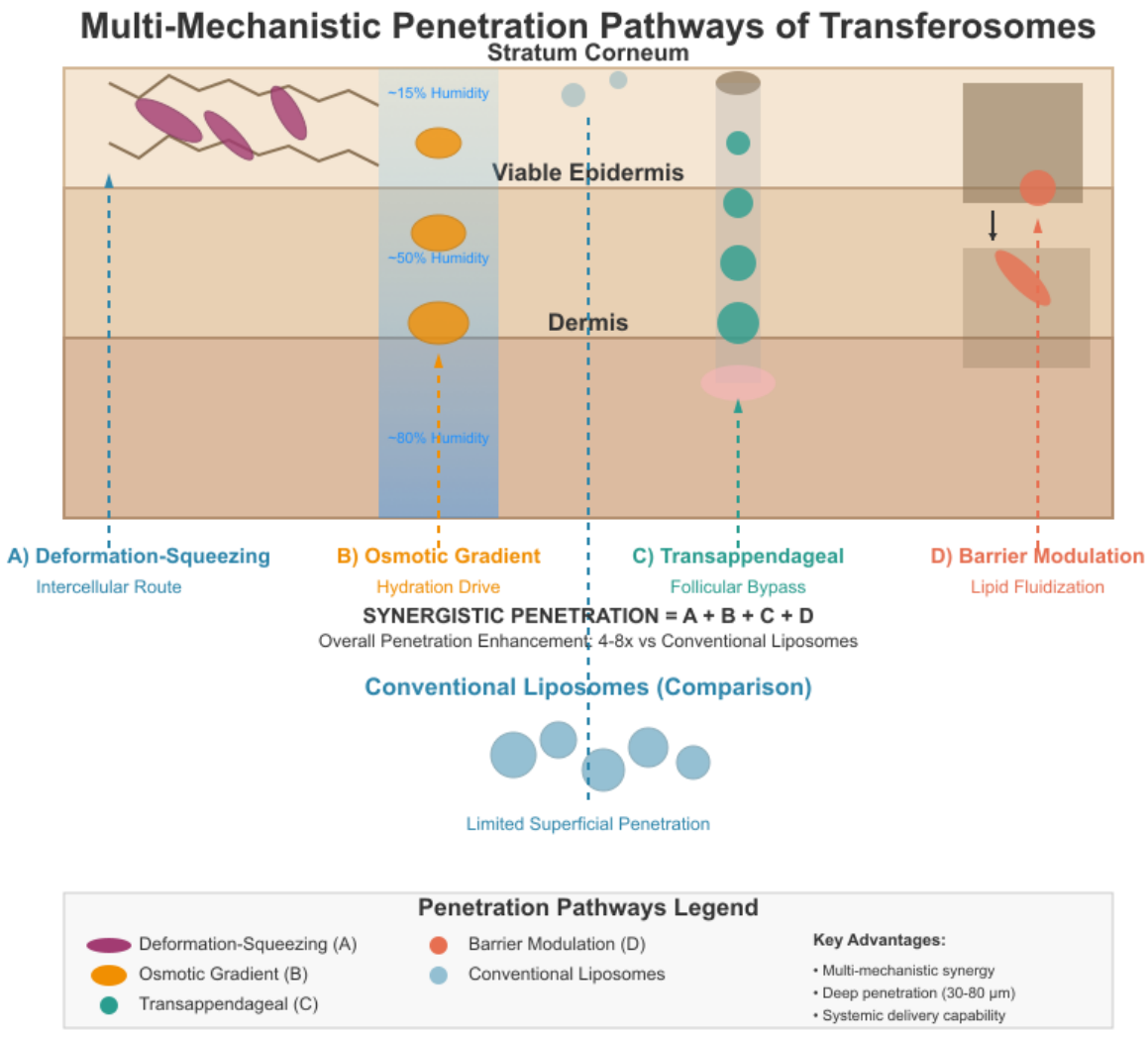

Figure 3: Multi-mechanistic penetration pathways of transferosomes[81 101]

3.6. Evidence and Validation

In Vitro Studies:

· Franz diffusion cells demonstrate superior flux rates

· Confocal microscopy shows deep dermal penetration with fluorescent markers

· SAXS analysis confirms membrane deformation during penetration[43 64 95].

Clinical Evidence:

· Insulin delivery with glycemic control equivalent to subcutaneous injection

· Vaccine applications showing robust immune response

· Analgesic formulations providing sustained pain relief

Recent Research Insights: "The combination of intrinsic deformability and extrinsic hydration response creates a unique 'push-pull' penetration dynamic that transcends conventional diffusion limitations." - Recent findings from skin penetration studies (2023)[65]

Transferosomes enable superior transdermal drug delivery through a sophisticated, multi-mechanistic strategy. Their key advantages include dynamic adaptability, allowing them to penetrate despite being larger than skin pores, and active, environmentally-responsive migration. They intelligently interact with the skin barrier, utilizing multiple pathways and creating a synergistic effect where the combined mechanisms exceed individual contributions[4 67 113]. This advanced functionality allows transferosomes to achieve delivery efficiencies once only possible with physical enhancers, positioning them at the forefront of non-invasive drug delivery technology[9 12 15].

4. PRODUCTION METHODS: FROM LABORATORY TO INDUSTRIAL SCALE

Manufacturing transferosomes requires precise control over composition and membrane properties to ensure consistent performance.

4.1. Conventional Methods

- Thin-Film Hydration: The classical Bangham method. It is simple but often results in heterogeneous vesicles and has limited scalability[67 94 145].
- Reverse-Phase Evaporation: Offers higher encapsulation for hydrophilic drugs, but complete removal of residual solvents is challenging[67].

4.2. Advanced Manufacturing Platforms

- Microfluidics: Enables precise control over mixing, producing monodisperse vesicles with tunable sizes. Its potential for continuous manufacturing and in-line monitoring makes it highly suitable for scale-up[16 69 115].
- Membrane-Based Technologies: Hollow fiber systems allow for scalable, reproducible production under gentle conditions, ideal for thermolabile compounds[70 114].

4.3. Emerging Approaches

- Supercritical Fluid Technology: A solvent-free method using supercritical CO_2 that produces uniform vesicles with enhanced stability and no organic solvent residues[71 93].
- Dual Asymmetric Centrifugation: A high-energy, solvent-free technique that generates small, uniform vesicles in a single step, showing promise for personalized medicine[72 76].

Table 2: Comparative Analysis of Transfersome Production Methods[4 74 75 76 112]

Method	Principle	Advantages	Limitations	Suitability/ Applications
Thin Film Hydration	Lipid and surfactant mixture is dissolved in an organic solvent, evaporated to form a thin film, and hydrated with aqueous phase to produce vesicles.	Simple, widely established, allows high encapsulation of lipophilic drugs.	Time-consuming; requires rotary evaporator; variable vesicle size distribution.	Preferred for small to medium-scale laboratory studies.
Reverse Phase Evaporation	Organic and aqueous phases are emulsified; removal of solvent leads to vesicle formation with drug entrapment.	High encapsulation efficiency for hydrophilic drugs; produces unilamellar vesicles.	Involves organic solvents (toxic risk); labor-intensive.	Useful for hydrophilic macromolecules (proteins, peptides).
Ethanol Injection	Lipid solution in ethanol is injected into aqueous phase, leading to spontaneous vesicle formation.	Simple, reproducible, avoids high-temperature processing.	Residual solvent removal essential; lower entrapment efficiency.	Suitable for thermo-labile drugs and rapid screening.
Sonication/ Extrusion	Large vesicles are downsized by probe sonication or passing	Produces uniform, nanosized vesicles; scalable.	Risk of lipid degradation due to heat and shear; possible metal	Commonly used for size reduction in

	through polycarbonate membranes.		contamination from probe.	formulation optimization.
Microfluidic Technique	Controlled mixing of lipid and aqueous streams in microchannels generates transfersomes with defined size.	Highly reproducible, scalable, precise size control.	Requires specialized equipment; high cost; limited availability.	Promising for industrial-scale, reproducible production.
Freeze–Thaw Cycling	Vesicles undergo repeated freezing and thawing, promoting fusion and reorganization.	Improves encapsulation of hydrophilic compounds; enhances stability.	Multiple cycles required; may destabilize sensitive drugs.	Applied as a secondary method to improve entrapment and vesicle stability.

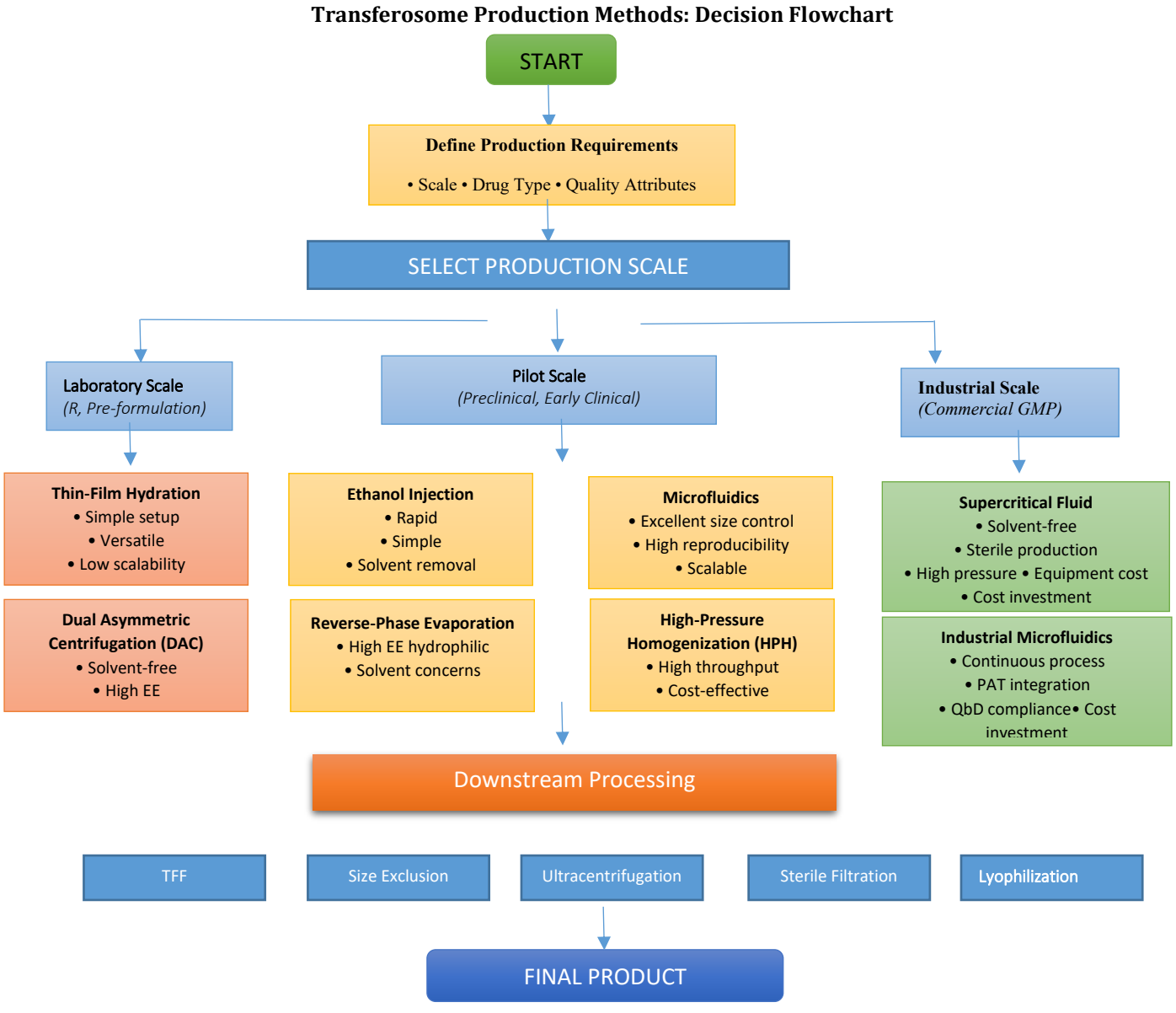

Figure 4: Flowchart showing transferosome production methods[23 58].

5. COMPREHENSIVE CHARACTERIZATION TECHNIQUES

Robust characterization is essential for establishing critical quality attributes (CQAs) and ensuring batch-to-batch consistency.

5.1. Size and Morphology

Dynamic Light Scattering (DLS): Measures hydrodynamic diameter and polydispersity index (PDI), with optimal values of 100-300 nm and PDI <0.3[77 111].

Nanoparticle Tracking Analysis (NTA): Provides accurate particle concentration and detects subpopulations by tracking individual vesicles[12 144].

Cryo-TEM: Reveals ultrastructural details like lamellarity and membrane integrity without staining artifacts[21 73].

5.2. Deformability Assessment

This defining characteristic is evaluated using specialized methods:

Pressure-Driven Filtration: Quantifies deformability by measuring the percentage of vesicles that permeate a membrane with pores smaller than their diameter[78 143].

AFM-Based Nanoindentation: Uses atomic force microscopy to measure nanoscale membrane elasticity (Young's modulus), which correlates with penetration efficiency[22 23 7 110].

5.3. Stability and Performance

Zeta Potential: Values between -20 mV and -30 mV indicate colloidal stability via electrostatic repulsion.

Drug Release Profiles: Assessed using Franz diffusion cells with synthetic membranes or ex vivo skin models.

Storage Stability: Evaluated through accelerated studies at various temperatures (e.g., 4°C, 25°C, 40°C) to monitor physical and chemical stability over time[24 25 109].

6. SCALE UP AND MANUFACTURING CONSIDERATION

The transition from laboratory to industrial-scale production is the primary challenge in commercializing transferosomes. Maintaining critical quality attributes (CQAs) like consistent particle size (PDI < 0.3), deformability, and high drug encapsulation requires robust engineering and process controls.

Key Challenges & Scale-up Strategy:

Major hurdles include preserving vesicle integrity, ensuring batch-to-batch uniformity, managing organic solvents, and guaranteeing sterility. A Quality by Design (QbD) framework is essential, defining a target product profile (e.g., size: 100-200 nm, encapsulation >80%) and identifying Critical Process Parameters (CPPs) for control.

- Formulation CPPs: Lipid concentration, edge activator ratio, and drug-to-lipid ratio are highly scale-sensitive and must be tightly controlled.
- Process CPPs: Laboratory methods (vortexing, rotary evaporation) must be replaced with controlled industrial processes like high-shear mixing and Tangential Flow Filtration (TFF), monitored in real-time using Process Analytical Technology (PAT).

Scalable Manufacturing Technologies:

- Microfluidic Systems: Enable continuous production with precise CQA control and easy PAT integration via parallelized chips.
- High-Pressure Homogenization (HPH): Effective for controlling particle size, requiring optimized pressure and temperature control.
- Supercritical Fluid Technology: A solvent-free, "green" alternative that offers simultaneous sterilization.

Downstream Processing & Quality:

Purification is efficiently scaled using TFF, while sterilization typically employs 0.22 µm filtration or integrated aseptic processing. Full Good Manufacturing Practice (GMP) compliance is mandatory, including detailed documentation and process validation for regulatory submissions (CMC section).

Economic & Future Outlook:

High costs are driven by raw materials, GMP equipment, and quality control. Cost reduction strategies include continuous manufacturing and process automation. Future trends point towards AI-optimized processes, continuous flow chemistry, and evolving regulatory pathways for streamlined nanomedicine approval.

Table 3: Formulation-Dependent Parameters and Roles[18 19 79]

Parameter	Optimal Range	Impact on Critical Quality Attributes (CQAs)	Scale-up Sensitivity
Lipid Concentration	10–50 mM	Directly influences vesicle diameter and entrapment efficiency of the active compound.	**High** – precise control over lipid hydration and mixing is essential during scale-up.
Edge Activator Ratio	10–25% w/w	Governs bilayer elasticity, deformability, and long-term stability of vesicles.	**Very High** – small deviations significantly alter transfersome flexibility and performance.

Aqueous Phase Composition	pH 6.5–7.4	Modulates drug stability, zeta potential, and vesicle surface charge characteristics.	**Medium** – buffer selection and volume scaling can pose reproducibility challenges.
Drug-to-Lipid Ratio	1:5 to 1:20	Affects drug entrapment efficiency, release kinetics, and formulation uniformity.	**High** – critical for batch-to-batch reproducibility in large-scale production.

Table 4: Process- Dependent Parameters and Role[18 20 81 141]

Parameter	Laboratory Scale	Industrial Scale	Control Strategy
Mixing Intensity	Typically achieved by manual shaking, vortexing, or probe sonication.	Controlled shear mixing using homogenizers or continuous flow systems.	Computational fluid dynamics (CFD) modeling and process analytical technology (PAT) ensure reproducible mixing.
Temperature Control	Maintained within ±2 °C using water baths or small incubators.	Precise regulation within ±0.5 °C using jacketed reactors and automated control units.	Real-time feedback loops and automated sensors stabilize process temperature.
Hydration Time	30–60 minutes, often dependent on manual agitation.	5–15 minutes, accelerated by high-shear mixers or automated hydration systems.	Process optimization algorithms minimize hydration variability.
Solvent Removal	Carried out by rotary evaporation under reduced pressure.	Achieved via tangential flow filtration (TFF) or spray drying for continuous processing.	Online solvent monitoring systems maintain residual solvent limits and ensure compliance.

7. REGULATORY AND COMMERCIAL LANDSCAPE

7.1. Regulatory Framework

Transferosomes occupy a regulatory gray area with no dedicated guidelines. They are evaluated case-by-case under existing nanomedicine frameworks (EMA, FDA). Critical checkpoints include comprehensive characterization (size, deformability), manufacturing reproducibility, and thorough safety profiling due to enhanced skin penetration. Approval requires strong risk-benefit justification supported by robust preclinical and clinical data[36 37 38 40 84 92].

7.2. Commercial Success Stories

Despite hurdles, transferosomes have seen commercial success, primarily in dermatology and cosmeceuticals where regulations are less stringent. Key examples include:

- Transferulin®: A transdermal insulin formulation in clinical evaluation.
- DermaVir® Patch: A DNA-based HIV vaccine delivered via transferosomes.

Cosmeceuticals: Numerous marketed skincare products for anti-aging and moisturizing. These products serve as a faster route to market, while therapeutic applications in areas like diabetes and vaccination progress more slowly under stricter review[39 41 85 122].

7.3. Intellectual Property Landscape

Patent filings have accelerated, showing strong industrial interest. Key areas of innovation include novel edge activators, hybrid systems (e.g., with microneedles), green manufacturing methods, and expansion into new therapeutic areas like oncology and gene therapy. However, a dense patent landscape may pose freedom-to-operate challenges for new entrants[31 32 33 130 131 132].

8. COMPUTATIONAL AND AI DRIVEN ADVANCES

8.1. Molecular Dynamics (MD) Simulations

MD simulations provide molecular-level insights into transferosome behavior. They visualize lipid bilayer deformation, track edge activator redistribution, and predict optimal lipid compositions to enhance membrane fluidity and stability, complementing experimental data[35 36].

8.2. Machine Learning (ML) Applications

ML models reduce trial-and-error by capturing the non-linear nature of formulation. They accurately predict vesicle size, optimize encapsulation efficiency, and classify vesicle deformability, significantly accelerating the design cycle[34 90 133 134].

8.3. Explainable AI (XAI) in Formulation Design

XAI addresses the "black-box" nature of ML, providing actionable insights by identifying key variables driving performance and establishing causal relationships. This enhances both rational experimental design and regulatory acceptability for AI-developed formulations [41 42 43 123 124].

9. CHALLENGES AND FUTURE DIRECTION IN TRANSFEROSOME DEVELOPMENT

Despite their potential, the clinical translation of transferosomes faces significant multifaceted challenges.

Technically, scaling up production while maintaining consistent vesicle deformability and stability remains difficult, often requiring complex lyophilization[125 128]. Drug loading efficiency and controlled release profiles also need improvement[43 44 45 86 126]. Biologically, efficacy is hampered by inter-individual skin variability and a lack of robust predictive models (IVIVC), while long-term safety data and the irritation potential of components are concerns[46 47 103 104 148 149]. Regulatory pathways are unclear due to a lack of standardized quality controls, and the landscape is complicated by intellectual property thickets and high production costs that challenge commercial viability[48 49 87 88 105 107]. Finally, scientific gaps in fundamental understanding and analytical tools for real-time characterization, coupled with manufacturing infrastructure and global regulatory harmonization needs, complete the major hurdles to widespread adoption[50 51 52 89 91 106 127 128 129 130 150].

10. CONCLUSION

Transferosomes embody a paradigm shift in transdermal drug delivery, demonstrating how intelligent nanocarrier design can overcome the formidable skin barrier and enable non-invasive administration of both small molecules and complex biologics. Their development journey highlights remarkable achievements, from mastering barrier penetration and expanding therapeutic applications to evolving scalable manufacturing strategies and securing clinical validation in diverse areas such as pain management, hormone therapy, and vaccination. The maturation of this technology is evident through strengthened scientific understanding, QbD-driven manufacturing, advanced analytical methods, and regulatory adaptability, all of which have contributed to its translation into meaningful clinical outcomes. With clear advantages such as enhanced patient compliance, improved pharmacokinetics, reduced side effects, and accessibility for self-administration, transferosomes represent a patient-centric innovation with broad implications for pharmaceutical sciences, including the validation of nanomedicine, establishment of rigorous characterization standards, and the shaping of regulatory guidelines. Beyond current applications, they have inspired next-generation deformable nanocarriers, expanded therapeutic frontiers, and advanced fundamental insights into membrane dynamics and biological barrier interactions. As the pharmaceutical field increasingly moves toward minimally invasive, patient-friendly therapies, transferosomes stand as a testament to interdisciplinary innovation and persistence, delivering not only drugs but also proof of the transformative power of nanotechnology in redefining modern medicine.

Conflicts of Interest: The authors declare that they have no conflicts of interest related to the content of this manuscript.

Author Contributions: All authors have contributed equally to the conception, design, preparation, and compilation of the manuscript.

Source of Support: Nil.

Funding: The authors declare that this study has received no financial support from any organization or agency.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the findings of this study are available within the cited references.

Ethical Approval: Not applicable.

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