Ethosomal Versus Liposomal Vesicular Systems for Topical Curcumin Delivery: A Critical Review of Mechanistic Evidence, Analytical Methodology, and Regulatory Landscape

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Abstract

Background: Curcumin, a naturally occurring polyphenol with well-characterised anti-inflammatory and antioxidant properties, has attracted growing interest as a topical therapeutic agent for inflammatory dermatoses, wound management, and photoprotection. Two compounding physicochemical challenges limit its clinical development: near-complete aqueous degradation at physiological pH (approximately 90% loss within 30 minutes at pH 7.4, 37 °C) and limited passive permeation across the stratum corneum (SC). Vesicular colloidal carriers—conventional phospholipid liposomes and ethanol-enriched ethosomes—have been extensively investigated as delivery platforms to address these barriers.

Scope: This narrative review critically examines the published literature on curcumin-loaded liposomal and ethosomal formulations for topical application. It evaluates the comparative physicochemical performance and SC permeation enhancement attributed to each system, provides a mechanistic re-evaluation of confocal laser scanning microscopy (CLSM) data commonly cited in support of intact vesicle penetration, identifies a widespread methodological error in Franz diffusion cell studies involving standard PBS as the receptor phase, reviews the stability analytical requirements specific to ethanol-rich vesicular systems, and maps the current FDA, EMA, and ICH regulatory landscape for topical vesicular drug products.

Key Findings: The literature consistently demonstrates superior SC deposition and permeation enhancement for ethosomes relative to conventional liposomes, attributable primarily to ethanol-mediated fluidisation of SC intercellular lipids rather than intact vesicle transport. CLSM, as routinely

applied in skin permeation studies, tracks fluorescent probe distribution in fixed tissue and cannot confirm vesicle integrity during transport; this distinction has been conflated in a substantial proportion of published ethosome studies. Standard PBS (pH 7.4) is analytically invalid as a Franz diffusion cell receptor phase for curcumin due to near-complete degradation within the experimental timeframe. Headspace GC-FID and Karl Fischer titration are identified as essential validated methods for ethanol content and moisture quantification in ethosomal stability programmes. No FDA- or EMA-centrally approved topical liposomal or ethosomal medicinal product currently exists, leaving regulatory development without a direct precedent.

Conclusion: This review identifies three correctable gaps in the published ethosome–curcumin literature—mechanistic misinterpretation of CLSM data, invalid receptor phase selection, and inadequate stability characterisation—and provides a framework for addressing each. It further positions topical curcumin vesicular formulations within the applicable regulatory framework, with specific guidance on CMC package requirements.

Keywords: *ethosome; liposome; curcumin; topical drug delivery; stratum corneum; Franz diffusion cell; CLSM; headspace GC-FID; Karl Fischer titration; critical quality attributes; bicyclopentadione; regulatory guidance; skin permeation; phospholipid vesicle*

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1. Introduction

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; MW 368.38 g/mol; $\log P \approx 3.29$) is a polyphenolic compound isolated from the rhizome of Curcuma longa L. that has accumulated a substantial evidence base for anti-inflammatory, antioxidant, antimicrobial, and wound-healing activity relevant to dermatological applications [1,2,3]. Clinical interest in topical curcumin spans inflammatory dermatoses—including psoriasis, atopic eczema, and contact dermatitis—wound management, and photoprotection [4,5]. Despite this pharmacological rationale, no curcumin-containing product has achieved regulatory approval as a topical drug in any major jurisdiction, in part because the compound's physicochemical properties create compounding formulation challenges that the published literature has not always addressed rigorously.

The two principal barriers are well established. First, curcumin's BCS Class IV classification—aqueous solubility < 0.6 µg/mL at neutral pH—means that only a small dissolved fraction is available for percutaneous absorption from any aqueous-based formulation [6]. Second, and less widely appreciated in the experimental literature, curcumin undergoes rapid degradation in aqueous environments at near-physiological pH: at pH 7.2–7.4 and 37 °C, approximately 90% of dissolved curcumin is lost within 30 minutes through alkaline hydrolysis and subsequent autoxidation of its β-diketone moiety [7,8]. The principal terminal degradation product is bicyclopentadione (BCP), formed via sequential spiroepoxide and vinyl ether intermediates, with secondary products including ferulic acid, vanillin, and acetovanillone [9]. Phosphate-buffered saline (PBS) at pH 7.4 has been identified as a particularly aggressive degradation medium, accelerating autoxidation relative to other buffers at equivalent pH [10]. These stability characteristics carry direct and underappreciated methodological consequences for in vitro permeation testing.

Phospholipid vesicular carriers—liposomes and ethosomes—represent the most extensively investigated drug delivery strategy for overcoming both the solubility and permeation limitations of curcumin for topical application [11,12]. Conventional liposomes, first described by Bangham, Standish, and Watkins in 1965 while studying ion diffusion across swollen phospholipid lamellae, are self-assembled bilayer vesicles that can encapsulate both lipophilic and hydrophilic drugs and have yielded numerous clinically approved parenteral products [11,12,13]. Applied topically, however, conventional liposomes demonstrate limited penetration beyond the superficial SC layers, constrained by bilayer rigidity and the unfavourable thermodynamics of intact vesicle entry into the dense SC intercellular lipid domain [1,30]. Ethosomes, introduced by Touitou and colleagues in 2000, incorporate high concentrations of ethanol (20–45% w/w) to produce softer, more deformable vesicles with substantially enhanced SC penetration profiles [14].

The published literature on curcumin-loaded ethosomes and liposomes is extensive but uneven in methodological quality. Three recurring problems are identifiable across a substantial proportion of published studies: (i) mechanistic claims of intact vesicle penetration derived solely from CLSM fluorescence data, which the imaging modality cannot support; (ii) use of standard PBS pH 7.4 as the Franz diffusion cell receptor phase, which causes near-complete curcumin degradation and renders permeation data analytically meaningless; and (iii) inadequate stability methodology for ethanol-rich vesicular systems, specifically the failure to independently quantify ethanol content and moisture by validated methods. This review systematically addresses each of these issues, provides a comparative framework for evaluating liposomal and ethosomal curcumin formulations, and maps the regulatory landscape that any translational development programme must navigate.

2. Curcumin: Physicochemical Profile and Topical Delivery Challenges

2.1 Molecular Properties and BCS Classification

Curcumin exists as three principal tautomers in solution—the bis-keto and two enol forms—with the enol tautomer predominating in organic solvents [6]. Its molecular weight (368.38 g/mol) places it at the upper boundary of what is generally considered acceptable for passive SC permeation under the 500 Da rule of thumb [3], though its high lipophilicity (log P ≈ 3.29) is theoretically favourable for SC partitioning. The practical barrier is that curcumin's log P, while above unity, significantly exceeds the log P 1–3 window considered optimal for balanced SC partitioning and viable epidermis penetration [3]. Compounds with log P > 3 tend to be effectively sequestered in the SC lipid phase, limiting their redistribution into the viable tissue layers.

BCS Class IV classification—low aqueous solubility and low membrane permeability—was originally developed to predict oral bioavailability, and the relevant permeability parameter for topical application is percutaneous flux rather than intestinal permeability [6]. However, the aqueous solubility constraint is directly relevant: at neutral pH, curcumin solubility is below 0.6 µg/mL, and the thermodynamic activity driving passive diffusion from any vehicle is correspondingly low. Complexation, encapsulation, or co-solvent strategies are therefore necessary to deliver therapeutically relevant concentrations to the SC.

2.2 Aqueous Degradation Pathways

Curcumin's chemical instability in aqueous media at physiological pH is multi-mechanistic and has been characterised in detail by several groups. Wang et al. demonstrated pH-dependent first-order degradation kinetics, with a half-life of approximately 8–17 minutes at pH 7.4 and 37 °C, increasing dramatically as pH is reduced below 6.5 [8]. The degradation follows two parallel pathways: alkaline hydrolysis of the β-diketone moiety, producing ferulic acid as the major product [7], and autoxidation proceeding through a spiroepoxide intermediate to a vinyl ether and ultimately to bicyclopentadione as characterised by Gordon et al. using LC-MS/MS and NMR [9]. BCP itself is biologically active, and some anti-inflammatory activities previously attributed to curcumin may be partially due to this degradation product—a complication for pharmacological interpretation [35].

The buffer identity, not merely the pH, also plays a role. Phosphate ions catalyse the autoxidation arm of the degradation pathway, making PBS a particularly aggressive medium relative to HEPES or acetate buffers at equivalent pH [10]. This observation has direct and critical implications for Franz diffusion cell methodology, which is discussed in detail in Section 4.2. A summary of curcumin degradation rates and products under conditions relevant to topical permeation testing is provided in Table 2.

Table 2. Summary of curcumin aqueous degradation under conditions relevant to topical permeation studies (data compiled from cited published literature).

Condition	Approximate t½	Principal Products	Reference
PBS pH 7.4, 37 °C	8–17 min	Bicyclopentadione (BCP), ferulic acid, vanillin	[7,8,9]
PBS pH 6.5, 37 °C	~2 h	BCP (slower formation)	[8]
PBS pH 5.5, 37 °C	> 6 h	Minimal degradation	[8,32]
Phospholipid bilayer encapsulation	Substantially extended	Reduced aqueous degradation	[14,15,44]
UV exposure (any pH)	Minutes	Photodegradation products (distinct pathway)	[29,45]

2.3 Implications for Topical Formulation Strategy

The combination of low aqueous solubility, high SC lipid affinity, and rapid aqueous degradation defines the formulation problem for topical curcumin. An ideal carrier must: (i) solubilise or encapsulate curcumin at therapeutically relevant concentrations; (ii) protect the drug from hydrolytic and oxidative degradation; (iii) enhance partitioning and diffusion across the SC; and (iv) provide a controlled release profile sufficient for local therapeutic effect. Vesicular phospholipid carriers—particularly ethosomes for their combined permeation-enhancement and encapsulation properties—address each of these requirements more completely than simple solutions, gels, or emulsions [14,15].

3. Liposomes and Ethosomes: Comparative Formulation Framework

3.1 Conventional Liposomes

Conventional liposomes are spherical colloidal vesicles formed by the self-assembly of amphiphilic phospholipids—predominantly phosphatidylcholine species—into one or more concentric bilayers

surrounding an aqueous core [11]. Their physicochemical characteristics are extensively tuneable: size (50 nm to several micrometres), lamellarity (unilamellar or multilamellar), surface charge (anionic, cationic, or neutral depending on lipid composition), and bilayer fluidity (governed by the lipid phase transition temperature relative to the storage or application temperature) [12]. The thin-film hydration method, in which dried lipid films are rehydrated with aqueous drug solution and the resulting multilamellar vesicles are sized by extrusion, remains the most reproducible preparation route for pharmaceutical applications [22].

For curcumin encapsulation, the phospholipid bilayer interior provides a lipophilic environment that strongly favours drug partitioning from the aqueous phase [38]. Published entrapment efficiencies for curcumin in conventional liposomes typically range from 60–85%, depending on the lipid-to-drug ratio, lipid composition, and preparation method [14,18]. The key limitation for topical application is bilayer rigidity: at temperatures below the phospholipid phase transition temperature, the ordered gel-phase bilayer is mechanically stiff, limiting deformation and restricting penetration to the superficial SC layers. Cholesterol incorporation, commonly used to improve liposomal stability, further increases bilayer rigidity [12,13].

3.2 Ethosomes

Ethosomes, introduced by Touitou and colleagues in 2000, are distinguished from conventional liposomes by the inclusion of ethanol at concentrations of 20–45% w/w [14]. This single compositional difference has profound consequences for the physicochemical behaviour of both the vesicle and the SC it contacts. Ethanol reduces the bilayer bending modulus by intercalating between phospholipid acyl chains, disrupting their ordered packing and creating a more fluid, deformable membrane [15,19]. This fluidisation simultaneously reduces the mean vesicle diameter relative to conventional liposomes prepared at equivalent lipid concentrations, increases the accessible lipophilic volume within the bilayer (improving encapsulation of lipophilic drugs such as curcumin), and reduces the energy barrier to vesicle deformation [14,40].

At the SC interface, ethanol exerts a permeation-enhancing effect independent of the vesicle structure itself: it intercalates into ceramide and free fatty acid bilayers of the SC lamellar bodies, transiently disorders their tightly packed alkyl chain organisation, and increases the diffusion coefficients of both drug molecules and the deformable vesicle membrane within the SC intercellular channels [19,20]. The cold ethanol injection method—in which an ethanolic solution of phospholipid and drug is injected into pre-warmed aqueous phase under stirring—is the most widely used preparation approach, yielding vesicles in the 100–250 nm size range with ethanol content dependent on the injection parameters and the initial ethanol fraction of the formulation [14].

A comparative summary of key physicochemical properties and their mechanistic bases for conventional liposomes and ethosomes is provided in Table 1.

Table 1. Comparative physicochemical properties of conventional liposomes and ethosomes relevant to topical curcumin delivery (compiled from published literature).

Property	Conventional Liposomes	Ethosomes	Mechanistic Basis
Ethanol content (% w/w)	0–5	20–45	Bilayer fluidiser; critical excipient [14]
Typical Z-average diameter (nm)	100–200	100–250	Ethanol reduces bending modulus [14,15]
Bilayer rigidity	High (rigid)	Low (deformable)	Ethanol disrupts acyl chain packing [15,19]
Stratum corneum penetration depth	Superficial SC only	SC + viable epidermis	Ethanol-mediated lipid fluidisation [16,20]
Encapsulation efficiency for lipophilic drugs	Moderate	Higher	Expanded lipophilic bilayer volume [15,40]
Primary stability CQAs	Diameter, PDI, EE%, phospholipid oxidation	Diameter, PDI, EE%, ethanol content, moisture	Ethanol loss → rigidity; moisture → aggregation [23,28]

3.3 Critical Quality Attributes for Each System

For both vesicular systems, hydrodynamic diameter (Z-average), polydispersity index (PDI), and zeta potential are the primary particle characterisation CQAs, measured by dynamic and electrophoretic light scattering respectively [36,37]. Entrapment efficiency (EE%), determined by ultracentrifugation rather than dialysis or gel filtration for curcumin (to avoid re-equilibration artefacts from the drug's high lipophilicity), is the primary drug loading CQA [39]. In vitro drug release is an additional CQA relevant to the regulatory package for both systems.

For ethosomal formulations specifically, ethanol content and free water content emerge as unique and primary CQAs that are absent from the conventional liposome quality framework. Ethanol content directly governs bilayer deformability and hence SC permeation performance: published data indicate that ethanol concentrations below approximately 35% w/w are associated with a shift toward conventional liposome-like rigidity, substantially diminishing the permeation advantage [40]. Moisture content is a CQA because free water promotes Ostwald ripening, vesicle fusion, and aggregation, manifesting as increases in Z-average diameter and PDI [28]. These two CQAs cannot be independently quantified by loss-on-drying, which reports only the combined volatile fraction; headspace GC-FID and Karl Fischer titration are the required methods, discussed in Section 4.3.

4. Critical Methodological Framework

4.1 Mechanistic Interpretation of CLSM Data

Confocal laser scanning microscopy of fluorescent probe-loaded vesicles in skin cross-sections has become the standard visualisation tool in the ethosome literature, and the observation of greater fluorescence depth or intensity in ethosome-treated sections relative to liposome-treated sections has been widely cited as evidence of intact vesicle penetration into the SC. This interpretation is mechanistically unsupported and requires correction.

CLSM of fixed, cryosectioned skin tissue images the spatial distribution of fluorescent molecules at the time of fixation. It cannot distinguish between fluorescence arising from intact vesicle-associated probe, from probe molecules that have dissociated from the vesicle membrane and diffused freely through SC intercellular channels, or from probe deposited within SC corneocytes [16,17]. Two landmark studies established this limitation clearly. Kirjavainen et al. demonstrated that lipophilic fluorescent probes dissociate from liposomal membranes within the SC environment, generating CLSM distribution patterns indistinguishable from those of free probe controls [17]. Verma and Fahr showed that ethanol and phospholipid act cooperatively to enhance topical delivery through a bilayer-fluidisation mechanism, with probe distribution patterns consistent with ethanol-mediated SC lipid fluidisation rather than intact carrier permeation [16].

TEM of SC sections from ethosome-treated skin has occasionally revealed vesicle-like structures, and these observations have been cited as corroborating evidence for intact vesicle penetration [14]. However, the frequency of such observations is low (typically fewer than 10% of fields examined in published studies), morphological consistency is poor, and sample preparation artefacts—specifically osmium tetroxide fixation-induced membrane fusion and ethanol dehydration during tissue processing—cannot be excluded as explanations [18,30]. Cryo-TEM of unfixed, rapidly frozen skin sections is the technically appropriate method for assessing in-situ vesicle structural integrity and has not been applied in the majority of published ethosome studies.

The mechanistic consensus supported by the biophysical evidence is that ethanol—at the concentrations employed in ethosomes—intercalates into SC ceramide and free fatty acid bilayers, transiently reduces the diffusion activation energy within the SC intercellular matrix, and enables both free drug molecules and the deformable ethosomal membrane to penetrate more deeply than rigid conventional liposomes [15,19,20]. A secondary kinetic contribution from membrane deformability during traversal of narrowed intercellular channels is plausible, but this should not be equated with intact vesicle transport across the full SC thickness, and CLSM data cannot resolve this distinction. Investigators reporting enhanced fluorescence depth in CLSM studies as 'evidence of intact vesicle penetration' should revise this interpretation to 'evidence of enhanced probe molecule penetration consistent with ethanol-mediated SC fluidisation.'

4.2 Franz Diffusion Cell Receptor Phase Selection for Curcumin

A critical and pervasive methodological error in the published curcumin topical permeation literature is the use of phosphate-buffered saline at pH 7.4 as the Franz diffusion cell receptor phase. This practice is analytically invalid for curcumin and produces data that are not interpretable as permeation measurements.

As detailed in Section 2.2, curcumin has a degradation half-life of 8–17 minutes in PBS at pH 7.4 and 37 °C [7,8]. In a standard 24-hour Franz diffusion cell experiment with receptor sampling at 0.5, 1, 2, 4, 6, 12, and 24 hours, the vast majority of any curcumin that permeates the skin and enters the PBS receptor phase will have degraded before sampling. Detected UV absorbance or fluorescence at curcumin wavelengths from such experiments reflects the combined signal of intact curcumin (a minor fraction), BCP, ferulic acid, and other degradation products—not curcumin permeation per se. Studies in the

published literature reporting curcumin permeation data from PBS pH 7.4 receptor phases should be interpreted with significant caution.

A methodologically valid receptor phase for curcumin Franz diffusion studies requires: (i) a pH sufficiently low to substantially reduce degradation kinetics—pH 5.0–5.5 is appropriate and within the physiologically relevant range for the skin surface [8,32]; (ii) a solubilising agent to maintain sink conditions and prevent drug precipitation—hydroxypropyl-β-cyclodextrin (HPβCD) at 0.5% w/v increases apparent solubility through inclusion complex formation and confers additional chemical stabilisation [32]; (iii) an antioxidant to suppress the autoxidative degradation pathway—L-ascorbic acid at 0.1% w/v is effective [34]; and (iv) oxygen exclusion through de-aeration and continuous nitrogen sparging, given the oxygen-dependence of the autoxidation pathway [9]. The modified receiver should be validated to demonstrate ≥ 85% curcumin recovery over the full experimental duration at 37 °C before permeation data are collected. LC-MS/MS confirmation of receptor phase samples using MRM transitions for intact curcumin (m/z 367→177, $[M-H]^-$) and BCP (m/z 273→229) allows accurate mass balance assessment and distinguishes permeated intact drug from in-situ degradation products [9,35].

4.3 Stability Methodology for Ethosomal Formulations

An ICH Q1A(R2)-compliant stability programme for ethosomal formulations [23] must monitor the CQAs described in Section 3.3. Standard stability conditions—4 °C (refrigerated), 25 °C/60% RH (intermediate), and 40 °C/75% RH (accelerated)—apply, with analysis at T = 0, 1, 3, and 6 months as a minimum. The CQA panel should include hydrodynamic diameter, PDI, zeta potential, EE%, membrane integrity (fluorescent probe leakage), ethanol content, and water content.

Ethanol content quantification requires static headspace GC-FID, following the general approach of European Pharmacopoeia monograph 2.4.24 [25] and validated per ICH Q2(R1) [26]. The method uses a DB-WAX or equivalent polar capillary column, an appropriate internal standard (n-propanol at 0.10% v/v), and a five-point external calibration spanning the relevant range. Although ethanol is classified as an ICH Q3C Class 3 residual solvent with a permissible daily exposure of 50 mg/day [27], in ethosomal formulations it functions as a primary functional excipient and should be treated as a formulation CQA with a justified lower specification limit—typically ≥ 35–38% w/w, based on the demonstrated concentration-dependent relationship between ethanol content and bilayer deformability [40].

Water content must be determined by Karl Fischer titration per Ph. Eur. 2.5.12 and USP ⟨921⟩ [28] rather than loss-on-drying. Ethanol and water are both volatile and both present in substantial quantities in ethosomal formulations; loss-on-drying reports their combined loss and cannot distinguish between them. Volumetric or coulometric Karl Fischer methods provide selective water quantification regardless of ethanol content. The combination of ethanol depletion and water ingress under storage is the most important degradation pathway for ethosomal physical integrity: ethanol loss increases bilayer rigidity toward that of a conventional liposome, while water ingress drives osmotic vesicle swelling and aggregation [28,36,40]. Both effects manifest as increases in Z-average diameter and PDI on stability testing.

Additionally, the polyunsaturated fatty acid composition of soy phosphatidylcholine (predominantly linoleic acid, C18:2) renders conventional liposomal formulations susceptible to phospholipid autoxidation

on storage. Stability programmes for liposomal formulations should therefore include a phospholipid oxidation index—thiobarbituric acid reactive substances (TBARS) or hydroxy fatty acid quantification by LC-MS/MS—alongside the standard vesicle characterisation parameters [12,36]. This monitoring requirement does not apply to the ethosomal preparation in the same way, as the ethanol content itself acts as a partial antioxidant in the bilayer environment.

5. Comparative In Vitro Skin Permeation: Review of Published Evidence

5.1 Published Comparative Studies

The published literature consistently reports superior SC permeation and drug retention for curcumin-loaded ethosomes relative to conventional liposomes, though the magnitude of enhancement varies substantially across studies, reflecting differences in formulation composition, skin model, receptor phase, and analytical methodology. Enhancement ratios for cumulative permeation (ethosomes versus liposomes) of 2–5-fold are commonly reported, with SC retention enhancements in the range of 1.5–3-fold [14,15,18]. Reported entrapment efficiencies for curcumin are typically 70–90% for ethosomes and 60–80% for conventional liposomes, consistent with the expanded bilayer lipophilic volume hypothesis [14,15,38].

Porcine ear skin is the most widely used surrogate for human skin in in vitro permeation studies, accepted under OECD TG 428 [21], and is broadly considered the most predictive non-human model due to its histological similarity to human skin in terms of SC thickness, lipid composition, and appendageal density. Full-thickness or dermatomed porcine ear skin at approximately 400–500 µm is the standard preparation, with TEER verification (typically ≥ 1 kΩ·cm²) before use to confirm barrier integrity.

5.2 Methodological Confounds in the Published Literature

Several systematic confounds reduce the reliability of the cumulative published data on curcumin vesicular permeation. The receptor phase issue discussed in Section 4.2 is the most consequential: studies using PBS pH 7.4 as the receiver cannot have measured curcumin permeation quantitatively. A secondary confound is the CLSM interpretation issue discussed in Section 4.1: studies that cite CLSM depth profiles as evidence of the superiority of ethosomes through a vesicle-transport mechanism are drawing conclusions that the data cannot support.

A third confound specific to comparative studies is the equimolar drug loading assumption: ethosomes and liposomes often differ substantially in EE%, meaning that equal nominal drug concentration in the donor formulation may correspond to very different encapsulated drug fractions. Permeation comparisons are only interpretable when the encapsulated drug fraction is matched, or when both encapsulated and free drug are quantified separately in the donor compartment at T = 0. Studies that report only nominal drug concentration in the donor without EE% verification may overestimate or underestimate the relative performance of one system.

A fourth consideration is the role of the ethanolic solution control. Many comparative studies test ethosomes versus liposomes without including a matched ethanol-in-water solution at equivalent ethanol

concentration as a control arm. This control is essential to isolate the phospholipid vesicle contribution from the ethanol permeation-enhancement contribution. Studies that omit this control cannot determine whether the observed performance advantage of ethosomes over liposomes reflects the vesicular structure, the ethanol concentration, or their combination. Where this control has been included, the phospholipid component typically contributes an additional 1.5–2.0-fold enhancement over the matched ethanolic solution alone [16], consistent with the cooperative mechanism described by Verma and Fahr.

5.3 Optimised Methodological Approach

Based on the analysis above, a methodologically valid comparative permeation study for curcumin vesicular systems should include: (i) an ethanolic solution control at matched ethanol concentration to the ethosome formulation; (ii) a modified receptor phase (PBS pH 5.5, 0.5% HPβCD, 0.1% ascorbic acid, nitrogen-sparged) validated for ≥ 85% curcumin recovery over 24 hours; (iii) TEER verification of skin integrity before each experiment; (iv) LC-MS/MS confirmation of receptor phase samples to distinguish intact curcumin from degradation products; (v) full mass balance reporting including SC tape-strip, viable epidermis, dermis, and receptor phase fractions; and (vi) permeation parameter reporting that includes both Jss and tlag alongside cumulative permeation. CLSM data should be reported as 'probe distribution profiles' rather than evidence of vesicle penetration mechanisms, with the probe-to-drug physicochemical comparison explicitly stated.

6. Regulatory Landscape for Topical Vesicular Drug Products

6.1 Current Approval Status

A fundamental regulatory reality that is frequently misrepresented or omitted in the published vesicular drug delivery literature is that no FDA- or EMA-centrally approved topical (cutaneous) liposomal or ethosomal medicinal product currently exists [47,48,52]. All FDA-approved liposomal drug products are parenteral or pulmonary in route of administration—including liposomal doxorubicin (Doxil®), liposomal amphotericin B (AmBisome®), liposomal irinotecan (Onivyde®), and liposomal bupivacaine (Exparel®) [47,48]—and the regulatory pathways, quality expectations, and bioequivalence frameworks developed for these products are not directly transferable to topical applications. Preparations marketed in the EU sometimes colloquially described as 'liposomal gels' are, on examination of their CMC data, microemulsion-based or polymer-matrix formulations rather than classical bilayer vesicular systems [53]. This regulatory gap means that any development programme for a topical liposomal or ethosomal drug product must construct its regulatory strategy without precedent approval as a reference point.

6.2 FDA Framework

The FDA 2018 Guidance on Liposome Drug Products—Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation [46] establishes the most detailed regulatory framework currently available for liposomal drug products. Although developed primarily in the context of parenteral liposomes, its CQA expectations—particle size distribution, lamellarity, entrapment efficiency, in vitro drug release, and physicochemical stability—are directly applicable to topical liposomal development. For bioequivalence of topical drug products, the FDA has developed in vitro release testing (IVRT) and dermatopharmacokinetic (DPK) tape-stripping methodologies [50]; these have not been extended to vesicular topical formulations in a finalised guidance, reflecting unresolved

questions about whether in vitro tools can adequately capture the performance differences attributable to vesicle structure.

6.3 EMA Framework

The EMA Guideline on Quality and Equivalence of Topical Products (EMA/CHMP/QWP/708282/2018) [51], in effect since 2019, is the primary reference for topical drug product quality and bioequivalence in Europe. The guideline explicitly states that differences in vehicle structure—including the presence of drug-carrying vesicles or microemulsions—may fundamentally alter drug release and skin penetration and may require clinical endpoint bioequivalence data beyond in vitro testing. This has significant implications for both innovator development (requiring robust clinical pharmacodynamic or clinical endpoint data) and generic development (requiring demonstration that vesicle characteristics are equivalent to the reference product).

6.4 CMC Requirements for Topical Curcumin Vesicular Development

Curcumin is not an approved active pharmaceutical ingredient for any indication in the US, EU, or UK. Development of a curcumin-based topical drug product therefore requires full IND-enabling studies (US) or a Clinical Trial Application with a complete investigational medicinal product dossier (EU). The CMC package must address API characterisation including curcuminoid purity profile (HPLC-UV/LC-MS/MS), polymorphism, and particle size distribution; formulation development rationale under ICH Q8(R2) QbD principles [41]; and product specifications per ICH Q6A [42] referencing all relevant CQAs.

For an ethosomal topical formulation specifically, the CMC package should document: Z-average diameter and PDI with justified acceptance criteria; zeta potential; EE% by ultracentrifugation; in vitro drug release using a validated modified receptor phase; ethanol content (GC-FID; lower specification limit justified by deformability data); moisture content (Karl Fischer); vesicle integrity at defined stability time points; photostability per ICH Q1B [45]; and comparative in vitro performance versus a non-vesicular control. This documentation aligns with the FDA liposome guidance [46], the EMA topical equivalence guideline [51], and the ICH quality guidelines series. Table 3 summarises the current regulatory framework.

Table 3. Regulatory framework summary for topical vesicular drug product development (compiled from cited regulatory guidance documents).

Regulatory Body	Key Guidance Document	Relevance to Topical Vesicular Products	Gap / Challenge
US FDA	Liposome Drug Products CMC Guidance (2018) [46]	CQA expectations: size, lamellarity, EE%, in vitro release	No approved topical liposomal drug product; IVRT/DPK not extended to vesicular systems [47,50]
EMA	Guideline on Quality and Equivalence of Topical Products (2019) [51]	Physicochemical differences in vehicle require clinical BE data	No centrally authorised topical liposomal/ethosomal product [52]
ICH	Q1A(R2), Q1B, Q2(R1), Q3C, Q6A, Q8(R2)	Stability, photostability, analytical validation, QbD framework	ICH guidelines not vesicular-specific; sponsor must adapt
Indian CDSCO	New Drugs and Clinical Trials Rules 2019	IND/CTA required for new topical drug; AYUSH framework for botanical	No specific vesicular topical guidance published

7. Future Perspectives

Several directions offer meaningful opportunities to advance the field of topical curcumin vesicular delivery beyond the current state of the literature. First, the mechanistic question of whether the deformable ethosomal membrane traverses SC intercellular channels as an intact vesicle—rather than as a disassembled lipid-drug mixture—remains genuinely unresolved. Cryo-TEM of rapidly frozen, unfixed skin cross-sections, combined with correlative fluorescence microscopy using probes of defined log P values, would provide the orthogonal evidence that CLSM alone cannot supply. This mechanistic clarification matters for formulation design: if intact traversal does occur to any significant extent, then bilayer composition and deformability are design parameters for SC depth; if it does not, then ethanol concentration and the partition coefficient of the drug become the primary performance drivers.

Second, the receptor phase problem identified in this review—the analytical invalidity of PBS pH 7.4 for curcumin Franz diffusion studies—extends to many other phenolic and polyphenolic compounds with similar pH-dependent instability. A systematic methodological survey establishing appropriate receptor phase conditions for the broader class of bioactive polyphenols under investigation for topical delivery would have considerable practical value for the field.

Third, the regulatory gap identified in Section 6 is itself a research opportunity. A formal comparison of in vitro and in vivo skin penetration data for a topical vesicular formulation, with the data package structured to meet FDA and EMA CMC guidance expectations, would provide the precedent that regulatory agencies and the pharmaceutical industry currently lack. Given curcumin's established safety profile at topical doses and its activity in psoriasis models, it is a reasonable candidate for this translational role.

Finally, scale-up and manufacturing consistency for ethosomal formulations remain underexplored. The cold ethanol injection method is well established at laboratory scale, but maintaining ethanol content within a narrow specification (e.g., 40 ± 2% w/w) during continuous or large-batch manufacture, and demonstrating CQA consistency across batches, are process validation challenges with no published regulatory precedent. Process analytical technology (PAT) tools—online DLS for particle size monitoring, in-line GC for ethanol content—may offer pathways to real-time release testing for future ethosomal drug products.

8. Conclusions

This review has examined the published literature on liposomal and ethosomal curcumin formulations for topical application through three critical lenses: mechanistic interpretation of experimental data, analytical methodology, and regulatory positioning. The following conclusions are drawn.

Ethosomes consistently outperform conventional liposomes for SC permeation and drug retention of curcumin across the published comparative literature. The primary mechanism is ethanol-mediated

fluidisation of SC intercellular lipids, not intact vesicle transport. The phospholipid vesicle component contributes an additional permeation enhancement beyond ethanol alone, consistent with a cooperative ethanol-phospholipid mechanism. These mechanistic conclusions are supported by biophysical evidence and by the comparative performance of matched ethanolic solution controls in the studies that have included them.

CLSM fluorescence depth profiles—the most commonly cited evidence for intact ethosome penetration in published studies—track probe molecule distribution in fixed tissue sections and cannot confirm vesicle integrity during transport. This methodological limitation has been conflated with mechanistic evidence of intact vesicle penetration in a substantial proportion of the published literature and should be corrected in future reporting.

Standard PBS at pH 7.4 is analytically invalid as a Franz diffusion cell receptor phase for curcumin, with near-complete drug degradation occurring within the experimental timeframe. A modified receiver—PBS pH 5.5, HPβCD, ascorbic acid, nitrogen sparging—validated at ≥ 85% curcumin recovery over 24 hours is the minimum methodological requirement. Published permeation data from PBS pH 7.4 receivers are not interpretable as curcumin permeation measurements.

Headspace GC-FID and Karl Fischer titration are the required analytical methods for independent quantification of ethanol and moisture in ethosomal stability programmes. Loss-on-drying is unsuitable for this application. Ethanol content and moisture are primary CQAs for ethosomal formulations with direct links to vesicle performance and physical stability.

No FDA- or EMA-centrally approved topical liposomal or ethosomal medicinal product currently exists. Development of topical curcumin vesicular formulations must proceed without approved precedent, drawing on the FDA liposome CMC guidance, the EMA topical equivalence guideline, and the applicable ICH quality guidelines series. Establishing rigorous methodological and regulatory foundations early in the development programme is the most effective strategy for avoiding late-stage CMC deficiencies.

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