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IN-VITRO STUDIES OF LIPOSOMAL A-LIPOIC ACID: FORMULATION STRATEGIES, PHYSICOCHEMICAL CHARACTERIZATION, RELEASE BEHAVIOR, ANTIOXIDANT ACTIVITY, CELLULAR INTERACTIONS AND THERAPEUTIC POTENTIAL

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ABSTRACT

α-Lipoic acid (ALA), also known as thioctic acid, is a naturally occurring organosulfur compound that functions as a cofactor in mitochondrial oxidative metabolism and possesses broad antioxidant and redox-regulatory properties. Both ALA and its reduced metabolite, dihydrolipoic acid (DHLA), participate in the regulation of reactive oxygen species, regeneration of endogenous antioxidants and maintenance of cellular redox homeostasis. Despite these pharmacological advantages, the therapeutic and nutraceutical application of ALA is constrained by limited aqueous solubility, rapid systemic elimination, extensive first-pass metabolism and formulation-dependent bioavailability. Liposomal delivery represents a potentially attractive strategy for overcoming several of these limitations because phospholipid vesicles can incorporate amphiphilic molecules within or near the lipid bilayer, protect labile compounds from environmental degradation and facilitate interactions with biological membranes. The present review critically examines the available in-vitro evidence for liposomal ALA, with emphasis on formulation development, lipid composition, preparation techniques, physicochemical characterization, encapsulation efficiency, structural organization, stability, release kinetics, antioxidant activity, cellular uptake, cytocompatibility, membrane interactions and application-specific performance. Evidence from conventional liposomes, chitosan-coated liposomes, phosphatidylcholine-based nanoforms and functional hybrid liposomes is discussed, while solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions and lipid nanocapsules are considered primarily for comparison. Published studies demonstrate that liposomal incorporation can modify the physicochemical behavior of ALA, promote sustained release in selected systems, enhance antioxidant performance and facilitate cellular delivery. Particularly promising findings have been reported for chitosan-coated ALA/CoQ10 liposomes, which demonstrated sustained release and enhanced skin accumulation, and for phosphatidylcholine/gelatin-curcumin hybrid liposomes, which showed rapid cellular uptake and prolonged ALA retention. A 2025 liposomal formulation designed for ocular administration further demonstrated enhanced DPPH radical-scavenging activity and cytocompatibility in human conjunctival epithelial cells. However, the evidence base remains fragmented, with considerable heterogeneity in lipid composition, particle size, analytical methods, loading approaches, release media and biological models. Moreover, direct comparisons between free ALA and equivalent-dose liposomal ALA remain limited. Future research should therefore prioritize standardized characterization, mechanistic cellular studies, physiologically relevant release models, quantitative uptake studies, long-term stability, scalable manufacturing and comparative pharmacokinetic evaluation. Collectively, current in-vitro evidence supports liposomal ALA as a promising formulation platform, but additional systematic investigation is required before definitive conclusions regarding superior bioavailability or clinical efficacy can be established.

KEYWORDS: α-lipoic acid; thioctic acid; liposomes; lipid nanocarriers; antioxidant; oxidative stress; drug delivery; encapsulation; controlled release; cellular uptake; in-vitro studies; nanomedicine.

1. INTRODUCTION

α-Lipoic acid (ALA; 1,2-dithiolane-3-pentanoic acid), commonly referred to as thioctic acid, is an organosulfur compound with a distinctive position at the interface of metabolism and redox biology. In its naturally occurring R-enantiomeric form, lipoic acid functions as an essential cofactor for mitochondrial multienzyme complexes involved in oxidative metabolism, particularly pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Beyond its metabolic function, ALA and its reduced form, dihydrolipoic acid (DHLA), have attracted extensive attention because of their ability to participate in antioxidant and redox-regulatory processes.[1–3] ALA can interact with several reactive oxygen species (ROS), while the ALA/DHLA redox couple can contribute to regeneration of other antioxidants and maintenance of intracellular redox balance.[1,2]

The antioxidant properties of ALA have stimulated investigation across numerous areas, including metabolic disorders, diabetes-associated complications, neurodegenerative diseases, cardiovascular disorders, ocular diseases, inflammatory conditions, skin aging and oxidative-stress-associated cellular injury.[2–5] However, the translation of these biological properties into optimized pharmaceutical and nutraceutical formulations is complicated by the physicochemical characteristics of the molecule.

Although ALA is readily absorbed following oral administration, its absolute bioavailability has been reported to be approximately 30%, largely because of extensive hepatic extraction.[6,7] The parent compound also exhibits relatively rapid plasma disappearance, with a reported half-life of approximately 25–35 min in pharmacokinetic studies of healthy volunteers.[6] These characteristics can limit sustained exposure and may influence the concentration of ALA available at specific biological targets. Consequently, considerable formulation research has focused on improving dissolution, protection, membrane interaction, controlled release and ultimately bioavailability.

Nanotechnology-based delivery systems have emerged as one approach to address these limitations. ALA has been incorporated into solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), nanoemulsions, lipid nanocapsules, polymeric nanoparticles, micelles, niosomes and liposomes.[8–11] A recent comprehensive review highlighted the diversity of these ALA delivery systems while emphasizing that the literature is considerably more extensive for non-liposomal lipid nanocarriers than for conventional liposomes.

Liposomes are particularly interesting because they consist of one or more phospholipid bilayers surrounding an aqueous compartment. Their amphiphilic architecture permits interactions with compounds of different polarity and provides a membrane-mimetic environment that may be advantageous for ALA. Importantly, ALA is itself amphiphilic and has an affinity for lipid environments. Recent physicochemical investigations have demonstrated that ALA can associate with phosphatidylcholine-based structures and influence their interfacial and membrane properties.[12]

Nevertheless, the specific in-vitro literature on liposomal ALA remains relatively limited. A 2020 review specifically examining liposomal antioxidants noted that research involving ALA-containing liposomes was considerably less developed than that involving other antioxidant delivery systems.

The objective of this review is therefore to critically evaluate the existing in-vitro evidence concerning liposomal ALA, with particular emphasis on the rationale for liposomal encapsulation of ALA; liposomal composition and formulation approaches; physicochemical characterization; encapsulation and drug loading; stability; in-vitro release; antioxidant activity; cellular uptake and intracellular retention; cytotoxicity and biocompatibility; membrane and skin interactions; disease-specific in-vitro models; comparison with other lipid nanocarriers; and current knowledge gaps and future research priorities.

2. Biological and Pharmacological Basis of α-Lipoic Acid

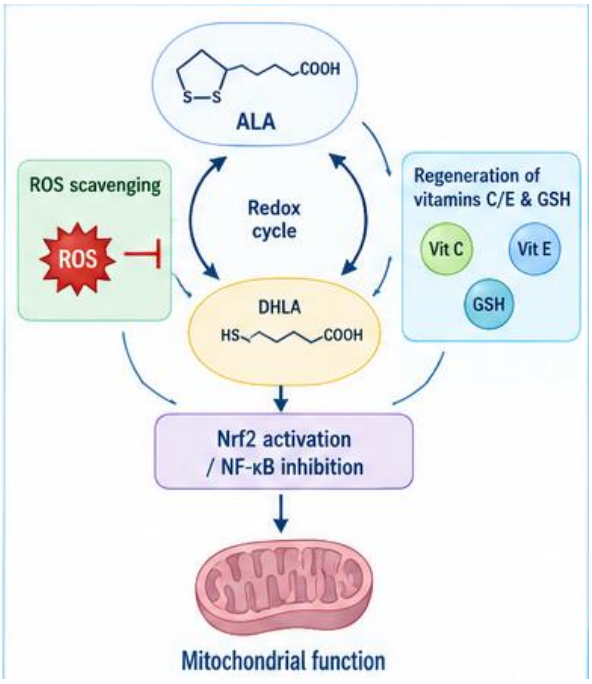


Figure 1: Biological Basis of ALA.

ALA is characterized by a five-membered 1,2-dithiolane ring attached to a pentanoic acid side chain. The oxidized molecule can undergo reversible reduction to DHLA, creating a biologically important redox couple. This reversible oxidation–reduction behavior contributes substantially to the biological activity of ALA.[1,2] The antioxidant properties of ALA are not attributable to a

single mechanism. Instead, ALA/DHLA participates in several interconnected processes involving direct radical scavenging, regeneration of endogenous antioxidants, modulation of cellular redox signaling and interaction with metal ions.[1,2] The foundational work of Packer et al. established ALA as an important biological antioxidant and described interactions with superoxide, hydroxyl radicals, hypochlorous acid, peroxyl radicals and singlet oxygen, as well as interactions with vitamin C, glutathione and vitamin E.[1]

ALA may influence intracellular antioxidant capacity through mechanisms extending beyond direct radical scavenging. The ALA/DHLA couple can contribute to the regeneration of antioxidant systems and influence glutathione-related redox processes. ALA has also been associated with modulation of redox-sensitive signaling pathways, including NF-κB and Nrf2-associated mechanisms.[2,3] This broad biological activity is particularly relevant to nanocarrier design. A delivery system that increases the concentration of intact ALA at the cellular interface may theoretically improve the probability of membrane interaction, cellular uptake and intracellular redox activity. Several formulation challenges provide the rationale for investigating liposomal ALA. ALA has relatively limited aqueous solubility, which complicates the development of highly concentrated aqueous formulations. A recent liposomal study reported an aqueous solubility of approximately 0.24 g/L and a log P of approximately 2.1, indicating appreciable affinity for lipid environments. Pharmacokinetic studies demonstrate rapid disappearance of ALA from plasma. Teichert et al. reported a short apparent half-life and approximately 29% absolute bioavailability following oral administration.[6,7] Although oral ALA is efficiently absorbed from the gastrointestinal tract, its systemic availability is restricted by extensive hepatic extraction. Teichert et al. estimated absolute bioavailability at approximately 30% following oral administration.[6] Different oral ALA formulations can produce substantially different plasma concentration–time profiles. Studies comparing conventional and modified-release formulations demonstrate that formulation architecture can alter Cmax and Tmax, highlighting the importance of delivery kinetics in determining systemic exposure.[7,13] These limitations provide a rational basis for designing lipid-based systems capable of modifying ALA's physicochemical environment and release profile.

4. Why Liposomes for α-Lipoic Acid?

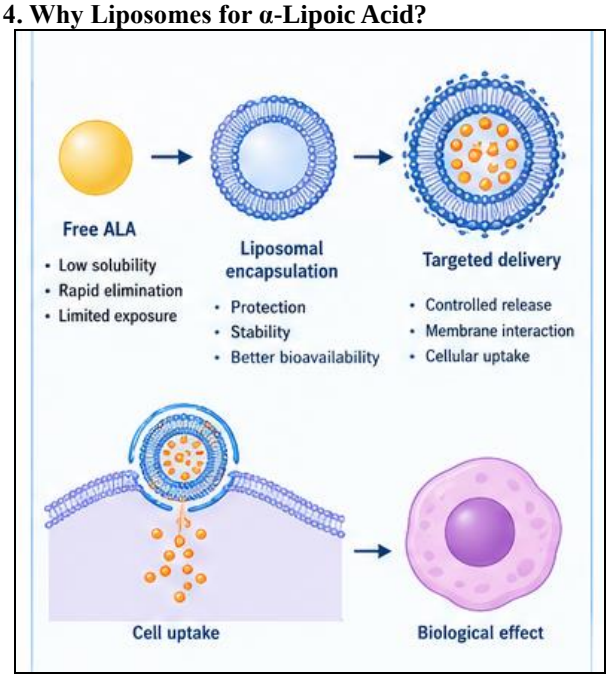


Figure 2: Mechanism of Liposomal Delivery of ALA.

Liposomes possess several characteristics that make them attractive for ALA delivery. First, the phospholipid bilayer provides a membrane-like hydrophobic environment in which ALA can associate through hydrophobic and polar interactions.[14] Second, liposomes can protect incorporated molecules from the external aqueous environment. Third, their surface can be modified using polymers such as chitosan or other functional molecules.[15] Fourth, liposomes can interact directly with cellular membranes and may facilitate intracellular delivery.[16]

The amphiphilic nature of ALA is particularly relevant. Rather than behaving simply as a water-soluble drug trapped inside the aqueous core, ALA can interact with the phospholipid membrane itself.[14-16] This distinction is important because loading efficiency and release kinetics may depend strongly on whether ALA is associated with the bilayer, located at the lipid–water interface or present within the aqueous compartment.[16]

Recent phosphatidylcholine-based investigations support this concept. Shchelkonogov et al. reported ALA-containing phosphatidylcholine nanoforms with particle sizes around 145 nm and high incorporation of ALA.[15] Cryo-TEM demonstrated both multilamellar and unilamellar structures, while release studies showed that approximately 45–55% of ALA had been released after 22 h. Interestingly, ALA release was initially faster from liposomes than from corresponding nanoemulsions.[16]

5. Methodology for Reviewing the Literature

For the present narrative review, literature was identified through searches of PubMed, PubMed Central and indexed scientific literature using combinations of the following terms.

- "alpha-lipoic acid"
- "lipoic acid"
- "thioctic acid"
- "liposome"
- "liposomal"
- "phosphatidylcholine"
- "hybrid liposome"
- "ALA encapsulation"
- "ALA release"
- "ALA cellular uptake"
- "ALA antioxidant"
- "ALA cytotoxicity"
- "liposomal alpha-lipoic acid"

Particular emphasis was placed on original studies reporting in-vitro formulation characterization, release, antioxidant activity, cellular uptake, cytotoxicity, membrane interaction or penetration.

Studies involving SLNs, NLCs, nanoemulsions, lipid nanocapsules and other nanocarriers were not considered equivalent to liposomes. Instead, they were used as comparative evidence to contextualize the performance and limitations of liposomal ALA.

Because the literature contains relatively few direct liposomal ALA studies, a narrative rather than quantitative meta-analytic approach was considered most appropriate.

6. Formulation Strategies for Liposomal ALA

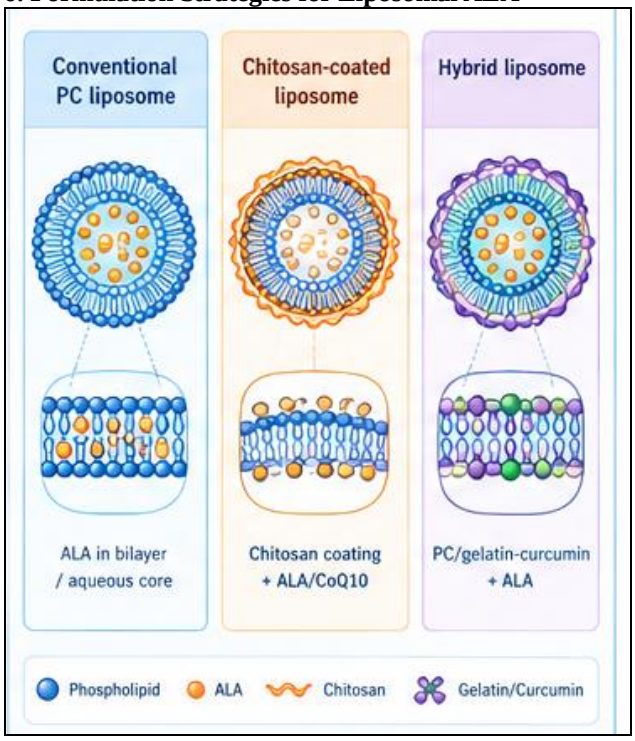


Figure 3: Types of Liposomal ALA.

Phosphatidylcholine is among the most commonly investigated lipid components for ALA delivery. Its amphiphilic structure enables spontaneous bilayer formation and provides a biologically compatible membrane environment.[17]

Shchelkonogov et al. investigated phosphatidylcholine-based ALA nanoforms and reported liposomal particles of approximately 145 nm. The formulations exhibited high ALA incorporation and remained stable during storage at room temperature. However, PDI values above 0.3 indicated some heterogeneity within the dispersions.[18]

This study is important because it provides direct evidence that ALA can be incorporated into phosphatidylcholine-based vesicular structures without destroying the fundamental organization of the liposome.[18]

A particularly important development was the creation of chitosan-coated liposomes containing both ALA and coenzyme Q10 (CoQ10).[17,18]

Zhao et al. developed spherical ALA/CoQ10 liposomes with a mean size of 183.4 ± 2.1 nm and PDI of 0.28 ± 0.03. The uncoated liposomes showed an ALA encapsulation efficiency of approximately 86.4%.[19] Chitosan coating increased the particle size and converted the surface from negative to positive. At the optimized 0.5% chitosan concentration, the particle size reached approximately 280 nm, while ALA encapsulation increased to approximately 88.6%.[19]

The proposed mechanism involves hydrogen bonding and ionic interactions between chitosan and the carboxyl-containing ALA molecule. The polymer coating therefore functions not merely as an external stabilizer but as an additional ALA-associated compartment.[20]

This formulation also demonstrated improved physicochemical stability, enhanced hydroxyl radical scavenging, sustained ALA release, and increased accumulation of ALA and CoQ10 in rabbit skin. These findings suggest that surface engineering can substantially modify the performance of ALA liposomes.[20]

7. Physicochemical Characterization of Liposomal ALA

Comprehensive characterization is essential because liposome performance is strongly influenced by size, PDI, surface charge, morphology, lipid composition and membrane organization. Particle size is one of the most frequently reported parameters.

Table 1: Published ALA liposomal systems demonstrate substantial variation.

Formulation	Approximate size	Major application
PC-based ALA nanoforms	~145 nm	General delivery[21]
ALA/CoQ10	183.4 ± 2.1	Skin

liposomes	nm	delivery[20,21]
Chitosan-coated ALA/CoQ10 liposomes	up to ~280 nm	Transdermal delivery[22]
LA@PCGC hybrid liposomes	~25 nm	Ototoxicity[22]
DPPC/POPC ALA liposomes	~404 nm	Ocular delivery[21,22]

Table 1 illustrates that there is no single "optimal" particle size for liposomal ALA. Instead, the appropriate size depends on the intended route and biological target. PDI provides information about particle-size distribution. Lower values generally indicate greater population homogeneity. The ALA/CoQ10 formulation exhibited a PDI of 0.28 ± 0.03, whereas the PC-based ALA nanoforms reported by Shchelkonogov et al. had PDI values above 0.3.[20] This difference illustrates an important methodological issue: particle size alone is insufficient to establish formulation quality. A formulation with an apparently favorable mean diameter may nevertheless contain a heterogeneous particle population.[20]

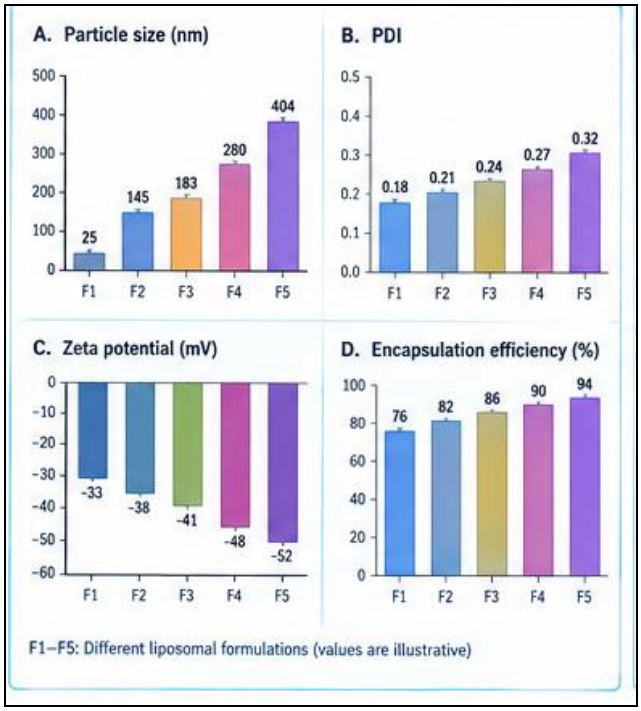


Figure 4: Physicochemical Characteristics of Liposomal ALA.

Surface charge affects colloidal stability, cellular interaction and biological distribution. The uncoated ALA/CoQ10 liposomes had a zeta potential of approximately −6.9 mV, whereas chitosan coating increased the surface charge to positive values, reaching approximately +29.4 mV at 0.5% chitosan.[21] The 2025 ocular liposome study similarly demonstrated a shift in zeta potential from +4.7 ± 0.4 mV for unloaded liposomes to −0.4 ± 0.3 mV after ALA incorporation. Such changes indicate that ALA can influence the surface characteristics of lipid vesicles and may alter interactions with biological membranes.[22]

TEM and cryo-TEM provide complementary information regarding vesicle morphology. The phosphatidylcholine-based ALA study demonstrated both multilamellar and unilamellar structures by cryo-TEM. Similarly, the 2025 ocular formulation retained spherical morphology following ALA incorporation, suggesting that ALA association with the lipid bilayer did not disrupt the overall vesicular structure.[21]

FTIR is particularly useful for investigating interactions between ALA and lipid or polymer components. In chitosan-coated ALA/CoQ10 liposomes, FTIR suggested hydrogen bonding and ionic interactions between ALA and chitosan.[22] In the 2025 ocular formulation, FTIR confirmed incorporation of ALA into the liposomal lipid bilayer. Incorporation also increased the bilayer transition temperature from approximately 37°C to 40°C. These observations are important because they suggest that ALA is not simply physically trapped within the vesicle but may influence lipid packing and membrane organization.[22]

Encapsulation efficiency is a critical parameter for evaluating liposomal ALA. The ALA/CoQ10 liposome system demonstrated an ALA encapsulation efficiency of approximately 86.4%, which increased to approximately 88.6% following optimization of chitosan coating.[22] The relatively high incorporation can be rationalized by the affinity of ALA for lipid interfaces and the possibility of secondary interactions with surface polymers.

However, encapsulation efficiency should not be interpreted independently of drug loading capacity. A formulation may exhibit high percentage encapsulation while containing only a small absolute quantity of ALA relative to the total lipid mass.[22]

Future studies should therefore report both.
EE (%) =encapsulated ALA/total ALA added×100
and
DL (%) =encapsulated ALA/total formulation mass×100
along with the analytical method used for ALA quantification.

10. In-Vitro Drug Release

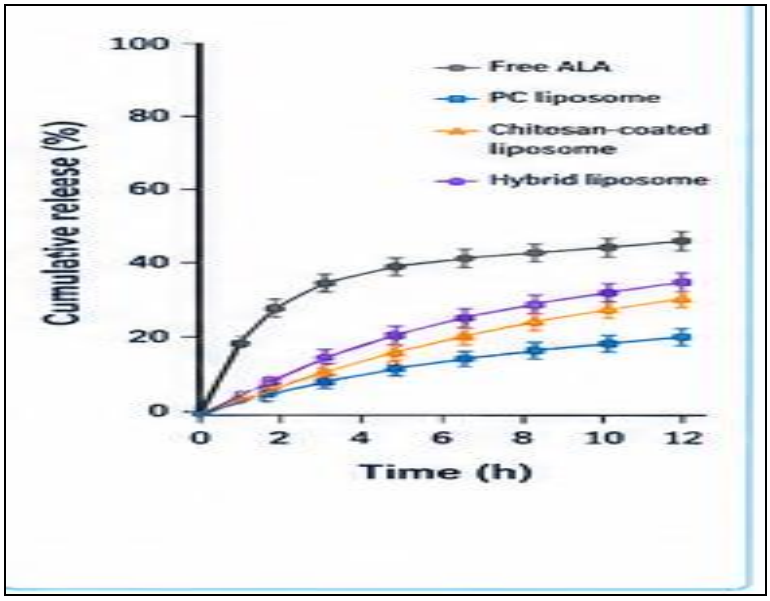


Figure 5: In-vitro Release Profiles of Liposomal ALA.

Drug-release behavior represents one of the most important advantages potentially offered by liposomal encapsulation. Shchelkonogov et al. reported that approximately 45–55% of ALA was released from phosphatidylcholine nanoforms after 22 h.[23] Interestingly, the initial release from liposomes was faster than from corresponding nanoemulsions. This finding illustrates an important point: liposomal encapsulation does not automatically mean slow release.[23]

Release kinetics depend on lipid composition, membrane fluidity, ALA–lipid interactions, lamellarity, particle size, temperature, pH, ionic strength, and composition of the release medium. Chitosan coating produced sustained ALA release in the ALA/CoQ10 system.[24] The likely explanation is the formation of an additional polymeric diffusion barrier surrounding the liposomal bilayer. Hydrogen bonding and electrostatic interactions between chitosan and ALA may further retard diffusion.[24] Thus, surface engineering can transform a conventional liposome into a more controlled-release delivery platform.

11. In-Vitro Antioxidant Activity

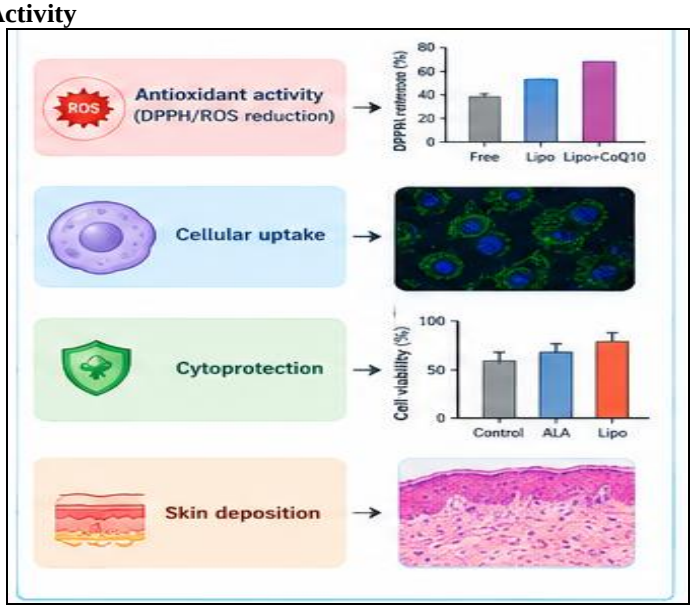


Figure 6: Antioxidant and cellular effects of liposomal ALA.

Antioxidant activity is arguably the most important biological endpoint for liposomal ALA. However, interpretation requires caution because antioxidant assays performed in solution do not necessarily predict intracellular antioxidant efficacy.[25] Several studies have investigated free ALA versus lipid-associated ALA using

chemical antioxidant assays. Zhao et al. demonstrated excellent hydroxyl radical-scavenging activity for chitosan-coated ALA/CoQ10 liposomes. More recently, Antezana et al. compared free ALA with ALA-loaded liposomes using the DPPH assay.[26] Free ALA at 5 mg/mL showed only approximately 5.3 ± 0.3% radical-scavenging activity, whereas the liposomal formulation containing approximately 4.03 mg/mL ALA demonstrated 31.6 ± 0.4% inhibition. Unloaded liposomes showed essentially no activity.[27] This represents a substantial increase in apparent antioxidant activity and suggests that the lipid environment can influence the accessibility of ALA to radical species.

Liposomal ALA Exhibit Enhanced Antioxidant Activity because of several mechanisms. Liposomal incorporation may improve the dispersion of poorly water-soluble ALA within an aqueous environment.[28] Because ALA associates with the lipid membrane, it may become concentrated at the lipid–water interface where it can interact with radical species. The liposomal environment may prevent aggregation of ALA and maintain it in a biologically accessible form. Phospholipid bilayers provide an environment resembling biological membranes, potentially facilitating subsequent interactions with cellular lipid domains.[27,28] Encapsulation may reduce exposure to environmental factors that could compromise ALA before it reaches its target. These hypotheses are consistent with the observation that liposomal ALA can display greater antioxidant activity than equivalent free ALA in some chemical assays. However, mechanistic cellular studies are still insufficient to establish which mechanism predominates.[28]

13. Cellular Uptake of Liposomal ALA

One of the strongest demonstrations of biological functionality comes from functional hybrid liposomes developed for cisplatin-induced ototoxicity. Curcio et al. developed hybrid liposomes composed of phosphatidylcholine and a gelatin–curcumin conjugate. The ALA-loaded formulation, termed LA@PCGC, had a mean diameter of approximately 25 nm.[29] Importantly, the formulation was rapidly internalized by HEI-OC1 auditory cells, with cellular uptake observed within approximately 1 h. ALA release from the hybrid liposomes remained below 20% even after 100 h, demonstrating a striking combination of rapid cellular uptake and prolonged intracellularly relevant retention potential.[30] This is particularly significant because the biological objective was not simply to increase ALA concentration in the extracellular environment but to deliver the antioxidant to cells exposed to oxidative injury. The LA@PCGC system provided one of the clearest examples of therapeutic in-vitro activity. HEI-OC1 cells exposed to 5 μM cisplatin exhibited cytotoxicity associated with oxidative stress.[30] Treatment with the ALA-containing hybrid liposomes improved cell viability, with the formulation increasing viability by up to approximately 2.19-fold relative to the relevant cisplatin-treated condition. This observation is important because it demonstrates that liposomal ALA can progress beyond physicochemical characterization and chemical antioxidant assays to a functional cellular model of oxidative injury.[29,30] The study further incorporated the liposomes into an alginate hydrogel intended for potential middle-ear application, demonstrating the possibility of combining nanovesicular delivery with a secondary biomaterial matrix.

15. Cytotoxicity and Biocompatibility

A successful liposomal antioxidant system must preserve the biological safety of both the carrier and the active compound.

The 2025 study by Antezana et al. evaluated liposomal ALA using IOBA-NHC human conjunctival epithelial cells. MTT analysis showed that both unloaded liposomes and ALA-containing liposomes permitted normal cellular growth at the tested conditions.[29] The authors therefore concluded that incorporation of ALA did not substantially compromise the cytocompatibility of the vesicles. These findings are particularly relevant to ophthalmic applications, where formulation tolerability is essential.[31]

The LA@PCGC hybrid liposome system also demonstrated functional compatibility with HEI-OC1 cells while protecting them against cisplatin-induced damage. Taken together, these findings indicate that ALA-containing liposomes can be engineered to achieve both biocompatibility and biologically meaningful antioxidant protection.[31]

16. In-Vitro Skin Penetration and Deposition

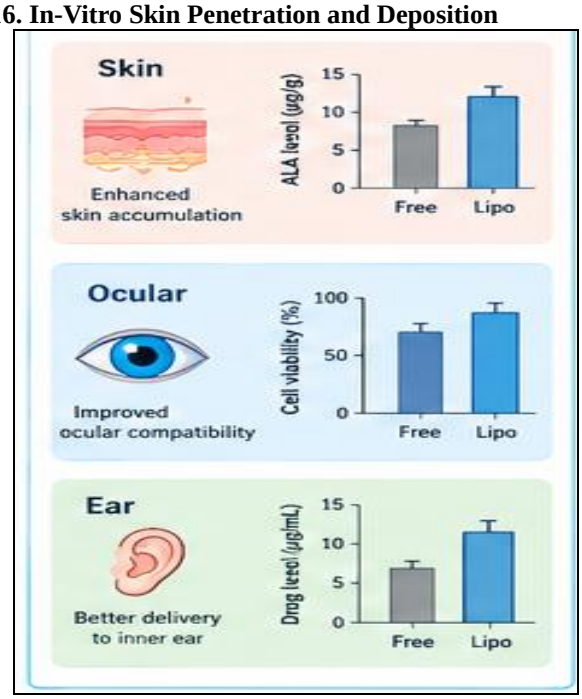


Figure 7: Application-specific performance of liposomal ALA.

The skin represents another important application for liposomal ALA. The chitosan-coated ALA/CoQ10 liposome developed by Zhao et al. was evaluated in rabbit skin. In-vitro penetration studies demonstrated enhanced accumulation of ALA and CoQ10 in the skin.[32] This finding is mechanistically plausible because liposomes can interact with the stratum corneum and lipid domains, while chitosan can increase residence time and modify surface interactions. Importantly, the objective for topical delivery is not necessarily complete transdermal passage. For antioxidant skin formulations, localized deposition within epidermal or dermal tissue may be more desirable than rapid systemic permeation.[31,32] Thus, the enhanced skin accumulation reported for the ALA/CoQ10 liposomal system may represent a desirable delivery outcome.

17. Liposomal ALA for Ocular Applications

Oxidative stress has an important role in several ocular disorders, including glaucoma. The 2025 study by Antezana et al. developed liposomes containing ALA specifically for potential ocular administration. The formulation was prepared using lipid-film hydration followed by extrusion.[30-32] The resulting ALA liposomes were approximately 404 ± 62 nm compared with 423 ± 36 nm for the unloaded liposomes. The formulation demonstrated preservation of spherical morphology, confirmed ALA incorporation into the bilayer, increased transition temperature, enhanced DPPH scavenging, and compatibility with human conjunctival epithelial cells. The study therefore provides a useful proof-of-concept for localized ophthalmic delivery.[27-30]

However, the study remains an early-stage formulation investigation. Additional studies involving corneal penetration, retinal delivery, tear-fluid stability, ocular residence time and disease-relevant retinal ganglion cell models would be necessary before therapeutic conclusions can be drawn.

18. Comparative Summary of Important In-Vitro Studies

Table 2: Summary of Important In-Vitro Studies of Liposomal ALA.

Study	Carrier	ALA formulation	Key characterization	Major in-vitro finding
Zhao et al.	Conventional/chitosan-coated liposome	ALA + CoQ10	183 nm uncoated; ~280 nm optimized coated system	Enhanced antioxidant activity, sustained release and skin accumulation
Shchelkonogov et al.	PC liposome/nanoform	ALA	~145 nm	High incorporation, long-term stability and measurable controlled release
Curcio et al.	Hybrid liposome	ALA + PC + gelatin-curcumin	~25 nm	Rapid cellular uptake, prolonged retention and protection against cisplatin
Antezana et al.	DPPC/POPC liposome	ALA	~404 nm	Enhanced DPPH activity and conjunctival-cell compatibility
Huang et al./related lipid systems	SLN/NLC/NE	ALA	~110–120 nm	Sustained release and antioxidant activity
Xia et al.	Lipid nanocapsule	ALA	~149 nm	High encapsulation, sustained release and skin deposition

The comparative evidence summarized in the Table 2 demonstrates that liposomal α-lipoic acid (ALA) has been investigated using diverse phospholipid architectures and surface-engineering strategies, with considerable differences in particle size, formulation composition and intended biological application. Despite these differences, a consistent finding across the available studies is that incorporation of ALA into lipid-based vesicular systems can substantially modify its physicochemical behavior and biological performance.[11] The earliest phosphatidylcholine-based studies established that ALA could be successfully incorporated into nanoscale lipid structures while maintaining vesicular organization. Shchelkonogov et al. reported phosphatidylcholine-based ALA nanoforms with a mean particle size of approximately 145 nm and demonstrated both unilamellar and multilamellar structures by cryo-TEM.[12] The formulation showed high ALA incorporation and measurable release over an extended period, with approximately 45–55% of the incorporated ALA released after 22 h. These findings provided important evidence that phosphatidylcholine can act as an effective lipid matrix for ALA and that the interaction between ALA and the phospholipid bilayer can influence its release behavior. Interestingly, the liposomal formulation displayed faster initial ALA release than the corresponding nanoemulsion, highlighting that the presence of a lipid component alone does not necessarily result in identical release kinetics and that vesicle architecture plays an important role in determining drug disposition.[13]

The development of chitosan-coated ALA/CoQ10 liposomes represented a further advancement in formulation engineering. Zhao et al. developed conventional ALA/CoQ10 liposomes with a particle size

of approximately 183 nm and a PDI of approximately 0.28, while subsequent chitosan coating increased the particle size to approximately 280 nm and substantially altered the surface charge.[14] The formulation exhibited high ALA encapsulation efficiency, which increased following optimization of the chitosan concentration. More importantly, chitosan modification influenced both the release characteristics and biological performance of the formulation. The coated vesicles demonstrated sustained ALA release and enhanced antioxidant activity, while skin-penetration studies indicated greater accumulation of ALA and CoQ10 in the skin compared with the corresponding non-liposomal formulations.[14] These findings suggest that surface modification can provide an additional level of control over liposomal ALA delivery. The positively charged chitosan layer may promote interaction with negatively charged biological surfaces, while the polymeric coating can act as an additional diffusion barrier, thereby influencing both residence time and release.

A substantially different approach was subsequently demonstrated by Curcio et al., who incorporated ALA into functional hybrid liposomes containing phosphatidylcholine and a gelatin–curcumin conjugate.[17] Unlike the relatively larger conventional liposomes described in previous studies, the resulting ALA-containing hybrid liposomes had an average diameter of approximately 25 nm. This small size was associated with rapid interaction with HEI-OC1 auditory cells, with cellular uptake detected within approximately 1 h.[17] At the same time, the formulation exhibited prolonged ALA retention, with less than 20% ALA release reported even after 100 h. This combination of rapid cellular uptake and prolonged release is particularly significant because it demonstrates that liposomal ALA can be designed not merely as a reservoir for extracellular antioxidant activity but as a vehicle capable of delivering ALA to cells subjected to oxidative stress. The functional relevance of this delivery was demonstrated using a cisplatin-induced ototoxicity model, in which the ALA-containing hybrid liposomes significantly improved cell viability compared with cisplatin-treated cells. Thus, this study provides one of the strongest examples of a direct relationship between liposomal ALA delivery and protection against a defined oxidative injury.

More recently, Antezana et al. investigated ALA-loaded DPPC/POPC liposomes for potential ophthalmic and neuroprotective applications.[18] In contrast to the very small hybrid liposomes reported by Curcio et al., these vesicles were approximately 404 nm in diameter. Despite their larger size, the formulation retained a spherical morphology following ALA incorporation and demonstrated measurable changes in surface charge and thermal properties, indicating interaction between ALA and the phospholipid bilayer.[18] Importantly, the ALA-loaded liposomes exhibited substantially greater DPPH radical-scavenging activity than free ALA under the experimental conditions employed. The formulation also demonstrated favorable cytocompatibility in human conjunctival epithelial cells, indicating that incorporation of ALA did not produce substantial cellular toxicity at the tested concentrations. These findings are particularly relevant because they extend the application of liposomal ALA from topical and general antioxidant delivery toward localized ocular delivery. Nevertheless, the larger particle size emphasizes that an optimal particle diameter cannot be defined independently of the intended route of administration and biological target.

When considered collectively, these studies demonstrate that liposomal ALA performance is highly dependent on formulation architecture. Particle size varied from approximately 25 nm in the functional hybrid liposomes to more than 400 nm in the ophthalmic formulation, while surface engineering ranged from unmodified phosphatidylcholine vesicles to positively charged chitosan-coated systems. Importantly, the biological outcome was not determined solely by particle size. The approximately 25-nm hybrid liposomes exhibited rapid cellular uptake, whereas the larger chitosan-coated liposomes were particularly effective for skin accumulation, and the approximately 400-nm ophthalmic liposomes demonstrated enhanced chemical antioxidant activity and cellular compatibility. Therefore, rather than identifying a universal optimal particle size, formulation development should consider the relationship between particle characteristics and the intended biological site of action.

The studies also reveal a progressive transition in the field from basic formulation development toward functional biological evaluation. Early investigations primarily focused on particle size, encapsulation efficiency, morphology, stability and release. Subsequent studies incorporated antioxidant assays and tissue-accumulation experiments, while more recent investigations have examined cellular uptake and protection against experimentally induced oxidative injury. This progression is important because successful nanocarrier development requires more than high encapsulation efficiency. A formulation must ultimately demonstrate that its altered physicochemical characteristics translate into improved biological availability or activity. The cisplatin-induced ototoxicity study is particularly valuable in this respect because it links formulation characteristics with cellular internalization, sustained ALA retention and a functional cytoprotective response.[17]

The comparative evidence also indicates that liposomal incorporation can enhance the apparent antioxidant activity of ALA, although the mechanism remains incompletely understood. The enhanced DPPH scavenging observed with the ocular liposomal formulation and the improved hydroxyl-radical-scavenging activity reported for chitosan-coated ALA/CoQ10 liposomes suggest that lipid association can

increase the functional accessibility of ALA.[14,18] However, chemical radical-scavenging assays should be interpreted cautiously because they do not necessarily reproduce the intracellular environment. The enhanced activity may reflect improved aqueous dispersion, localization of ALA at the lipid–water interface, changes in molecular organization or protection of ALA from degradation. Consequently, future investigations should complement chemical assays with intracellular ROS measurements, glutathione analysis, mitochondrial oxidative-stress assays and quantitative intracellular ALA/DHLA measurements.

An important observation from the comparative table is that most studies have evaluated different formulations for different biological purposes, making direct ranking difficult. The chitosan-coated ALA/CoQ10 system was primarily developed for topical application, the hybrid liposome was designed to protect auditory cells from cisplatin-induced injury, and the DPPC/POPC system was developed for potential ocular neuroprotection. Thus, the apparent differences in particle size, release rate and surface characteristics should be understood as application-specific design choices rather than simply differences in formulation quality. A formulation intended for rapid cellular uptake may appropriately be engineered differently from one intended to provide prolonged skin residence or localized ocular antioxidant activity.

The inclusion of non-liposomal lipid nanocarriers in the comparative literature further strengthens this interpretation. ALA-loaded solid lipid nanoparticles, nanostructured lipid carriers and lipid nanocapsules have frequently demonstrated high encapsulation efficiencies, nanoscale particle sizes, sustained release and antioxidant or cytoprotective effects.[9–11] However, these systems possess solid or semi-solid lipid matrices rather than the aqueous-core/phospholipid-bilayer architecture characteristic of liposomes. Their inclusion as comparative systems is therefore useful for establishing the broader potential of lipid-based ALA delivery but should not be used to imply that all lipid nanocarriers exhibit equivalent mechanisms of drug incorporation or release.

Overall, the comparative studies indicate that liposomal ALA is progressing from a primarily formulation-oriented research area toward a biologically validated delivery platform. Conventional phosphatidylcholine liposomes established the feasibility of ALA incorporation and controlled release, chitosan-coated systems demonstrated the advantages of surface engineering and enhanced tissue deposition, functional hybrid liposomes provided evidence for rapid cellular delivery and protection against oxidative injury, and more recent ophthalmic formulations demonstrated enhanced antioxidant activity together with cytocompatibility. Nevertheless, the small number of direct studies and substantial methodological heterogeneity prevent definitive conclusions regarding the superiority of one liposomal architecture over another. Future investigations should therefore employ standardized experimental conditions and directly compare free ALA with equivalent-dose liposomal ALA while simultaneously assessing physicochemical characteristics, release, cellular uptake, intracellular ALA/DHLA concentrations and functional oxidative-stress endpoints. Such studies would provide a more robust basis for identifying the formulation characteristics that are genuinely responsible for improved ALA delivery and biological efficacy.

19. Critical Evaluation, Translational Considerations and Future Perspectives

Although the available literature provides encouraging evidence for liposomal delivery of α-lipoic acid (ALA), the current evidence base remains relatively limited and heterogeneous.[18,19] A major limitation is the small number of studies that have specifically investigated true ALA-loaded liposomes compared with the much broader literature on other lipid- and nanocarrier-based systems.[18,19] A considerable proportion of the available nanotechnology literature on ALA involves solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions and lipid nanocapsules rather than conventional phospholipid liposomes.[18,19,24–28] These systems provide useful comparative evidence that lipid-based nanocarriers can improve the physicochemical and biological performance of ALA, although their structural organization and drug-release mechanisms differ from those of liposomes.[18,19,30–33] Consequently, findings obtained with non-liposomal lipid nanoparticles should be interpreted as supportive evidence for lipid-based delivery rather than direct evidence of the superiority of liposomal ALA.[18,19]

The heterogeneity of published liposomal formulations represents another important limitation. Differences in phospholipid composition, cholesterol concentration, ALA-to-lipid ratio, preparation method, particle size, surface modification and vesicle architecture can substantially influence encapsulation efficiency, membrane characteristics, stability and drug release.[20,21,30–33] Phosphatidylcholine-based ALA nanoforms, for example, have demonstrated nanoscale vesicle formation with measurable ALA incorporation and prolonged release characteristics.[20] Chitosan coating provides an additional formulation variable because it can modify particle size, surface charge, release behavior and interaction with biological surfaces.[21] These formulation-dependent differences make direct comparison among studies difficult and emphasize the importance of standardized reporting of critical formulation parameters.[18,19,30–33]

Release studies provide further evidence that liposomal encapsulation can modify the disposition of ALA. Phosphatidylcholine-based ALA nanoforms demonstrated sustained release over an extended

experimental period, indicating that association of ALA with the lipid vesicular structure can delay its liberation into the surrounding medium.[20] Chitosan-coated ALA/CoQ10 liposomes similarly demonstrated modified release behavior compared with non-coated formulations, suggesting that the polymeric surface layer can provide an additional diffusion barrier.[21] However, differences in dialysis membranes, release media, temperature, sampling schedules and analytical procedures among studies complicate direct comparison of reported release percentages.[18,19] Future investigations should therefore employ standardized and physiologically relevant release conditions appropriate to the intended route of administration.[18,19,30]

The biological evidence is particularly important because improved physicochemical characteristics alone do not establish enhanced therapeutic performance. ALA is a potent redox-active compound capable of participating in antioxidant and redox-regulatory processes, including regeneration of endogenous antioxidants and modulation of oxidative-stress pathways.[1–10] Conventional chemical assays such as DPPH and related radical-scavenging methods provide useful preliminary evidence but cannot fully reproduce intracellular antioxidant mechanisms.[18,19] The enhanced antioxidant activity reported for ALA-loaded liposomal systems may arise from improved dispersion, altered localization of ALA within or near the lipid membrane, protection against degradation or improved availability to reactive species.[18–21] Therefore, future studies should complement chemical antioxidant assays with cellular measurements of intracellular reactive oxygen species, glutathione status, lipid peroxidation, mitochondrial membrane potential and antioxidant enzyme activity.[1–10,18,19]

Direct comparison between free ALA and liposomal ALA remains an important research requirement. Although several studies have reported improved antioxidant activity, cellular uptake, sustained release or cytoprotective effects following liposomal incorporation, equivalent-dose comparisons are not consistently performed under identical experimental conditions.[20,21,23] Such comparisons are necessary to determine whether the observed improvement results from increased cellular delivery, prolonged exposure, altered intracellular localization or improved chemical stability.[18,19] Quantification of intracellular ALA and its reduced metabolite dihydrolipoic acid would provide particularly valuable mechanistic evidence linking formulation characteristics with biological activity.[1–3,5,9]

The available studies also demonstrate that liposomal ALA may have application-specific advantages. Chitosan-coated ALA/CoQ10 liposomes have been investigated for enhanced topical antioxidant delivery and demonstrated increased skin accumulation compared with corresponding non-liposomal systems.[21] Functional hybrid liposomes containing ALA have demonstrated rapid uptake by auditory cells and prolonged ALA retention, together with protection against cisplatin-induced cellular injury.[22] More recently, ALA-loaded DPPC/POPC liposomes have been investigated for potential ocular neuroprotective applications and demonstrated enhanced radical-scavenging activity together with favorable cytocompatibility in human conjunctival epithelial cells.[23] These findings indicate that liposomal ALA can potentially be adapted to different biological targets through modification of lipid composition, particle characteristics and surface properties.[21–23,30–34]

The variation in particle size among reported formulations further demonstrates that an optimal particle size cannot be defined independently of the intended application. Phosphatidylcholine-based ALA nanoforms were reported in the nanoscale range, whereas functional hybrid liposomes and ophthalmic formulations demonstrated substantially different dimensions.[20,22,23] Particle size can influence colloidal stability, cellular interaction, tissue distribution and biological uptake, but these effects are also strongly dependent on surface charge, membrane composition and biological environment.[30–33] Therefore, formulation optimization should focus on the overall relationship among particle size, PDI, surface characteristics, encapsulation, release and biological response rather than selecting particle size as an isolated quality attribute.[30–33]

The progression of the literature reflects a transition from basic formulation characterization toward increasingly sophisticated biological evaluation. Early work primarily established the feasibility of incorporating ALA into phospholipid-based nanosystems and characterized their physicochemical and release properties.[20] Subsequent investigations introduced surface modification and co-encapsulation with other antioxidants such as CoQ10, thereby demonstrating the possibility of improving tissue deposition and antioxidant performance.[21] Functional hybrid liposomes subsequently provided evidence of cellular uptake and protection against chemically induced oxidative injury.[22] More recent work has extended this concept toward ocular delivery, where enhanced antioxidant activity and cytocompatibility have been demonstrated in an in-vitro epithelial model.[23] Collectively, these developments indicate increasing recognition that successful ALA nanodelivery requires demonstration of functional biological benefit in addition to high encapsulation efficiency.[18,19]

From a translational perspective, manufacturing considerations will be important for converting laboratory-scale liposomal ALA formulations into reproducible products. Conventional liposome preparation methods such as thin-film hydration are valuable during laboratory formulation development, but scale-up can introduce challenges related to solvent removal, batch-to-batch reproducibility, vesicle-size control and sterilization.[30–33] Alternative approaches,

including ethanol injection, homogenization and microfluidic production, may provide improved control over particle characteristics and potentially facilitate continuous or scalable manufacturing.[30–33] Long-term stability must also be considered because phospholipid oxidation, aggregation, leakage and changes in vesicle structure can influence both formulation quality and ALA retention.[30–33]

A quality-by-design strategy may therefore be particularly useful for the systematic development of liposomal ALA. Particle size, PDI, zeta potential, ALA content, encapsulation efficiency, drug loading, lipid composition, release profile and chemical stability can be considered important quality attributes that require systematic control.[30–33] Establishing relationships between formulation composition, process parameters and biological performance could facilitate rational optimization and scale-up of liposomal ALA formulations.[30–33]

Future research should move beyond demonstrating that ALA can be incorporated into phospholipid vesicles and should instead establish the mechanistic relationship between liposomal architecture, ALA disposition, cellular uptake and biological response.[18,19] Advanced formulations incorporating surface-modifying polymers, targeting ligands or stimuli-responsive components may provide opportunities for improving site-specific delivery, although increased formulation complexity should be justified by demonstrable biological benefits.[18,19,30–33] Particular attention should be given to mitochondrial targeting because oxidative stress and mitochondrial dysfunction represent important components of several pathological processes in which ALA has demonstrated biological activity.[1–3,5,9]

Overall, the available evidence supports liposomal ALA as a promising lipid-based delivery strategy, particularly for applications requiring enhanced antioxidant activity, controlled release, cellular delivery or localized tissue deposition.[18–23] However, the relatively small number of direct liposomal ALA studies, differences in formulation architecture and substantial methodological heterogeneity currently prevent definitive conclusions regarding the superiority of one liposomal system over another.[18,19] Future investigations should therefore directly compare free ALA, conventional liposomal ALA and appropriately selected alternative nanocarriers under standardized experimental conditions.[18,19] Integrating physicochemical characterization, release kinetics, cellular uptake, intracellular ALA/DHLA quantification and functional oxidative-stress endpoints would provide stronger mechanistic evidence for the advantages of liposomal delivery.[1–3,18,19] Such studies will be essential for establishing whether the promising in-vitro findings obtained to date can ultimately translate into improved pharmacokinetic exposure, tissue distribution and therapeutic efficacy.

CONCLUSIONS

The available literature demonstrates that liposomal delivery represents a promising but still relatively underexplored strategy for improving the formulation and biological performance of α-lipoic acid. Unlike conventional free ALA, which is affected by limited aqueous solubility, rapid systemic disposition and formulation-dependent exposure, liposomal incorporation can modify the physicochemical environment of ALA, promote its association with phospholipid membranes and enable controlled interaction with biological interfaces.

In-vitro studies provide several lines of evidence supporting this approach. Phosphatidylcholine-based liposomes have demonstrated substantial incorporation and measurable release of ALA, while chitosan-coated ALA/CoQ10 liposomes have shown enhanced encapsulation, sustained release, antioxidant activity and skin accumulation. Functional hybrid liposomes have demonstrated rapid cellular uptake and prolonged ALA retention while protecting auditory cells against cisplatin-induced oxidative injury. More recently, ALA-containing liposomes designed for ocular applications demonstrated enhanced DPPH radical-scavenging activity and good compatibility with human conjunctival epithelial cells.

Nevertheless, the current evidence does not yet establish that all liposomal ALA formulations are inherently superior to free ALA. The literature is characterized by substantial heterogeneity in lipid composition, preparation technique, particle size, ALA loading, release conditions and biological models. Furthermore, the number of studies directly examining intracellular delivery, mitochondrial effects, redox signaling and disease-specific mechanisms remains limited.

The principal research priority should therefore shift from demonstrating that ALA can be incorporated into liposomes toward determining how liposomal architecture controls ALA disposition, intracellular delivery and biological activity. Standardized formulation characterization, equivalent-dose comparative studies, physiologically relevant release models and mechanistic cellular experiments will be essential.

Overall, the available in-vitro evidence supports liposomal ALA as a scientifically credible platform with potential applications in topical, ocular, neurological, auditory and systemic antioxidant delivery. However, additional well-controlled studies are required to establish reproducibility, mechanism, long-term stability, manufacturing scalability and translational superiority before the technology can be considered clinically validated.

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