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In-Vitro Characterization of Novel Liposomal Alpha Lipoic Acid Formulation by WBCIL for Enhanced Bioavailability

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ABSTRACT

The clinical use of Alpha Lipoic Acid (ALA), a potent antioxidant, is limited by its poor oral bioavailability due to low water solubility and rapid degradation. To address these issues, West Bengal Chemical Industries Ltd., Kolkata, India (WBCIL) developed a novel Liposomal ALA formulation using a spray-drying process. This study evaluates the physical and chemical properties of this formulation at different production scales, from a small R&D batch to larger commercial batches. The study focused on assessing morphological changes, elemental composition, and in-vitro release profiles. The results showed that the liposomal formulation significantly improved ALA release in simulated salivary fluid, with the large-scale spray-dried batches releasing 55% to 57% of ALA in one minute, compared to only 9% for normal ALA. The large-scale batches also demonstrated superior and consistent sustained release over two hours (42%) and complete release over four hours (86-89%) in dialysis studies, proving the formulation's controlled and efficient delivery. Furthermore, the liposomal batches, particularly the large-scale ones, showed enhanced oxidative stability, recovering 67% of ALA after two hours, compared to 25% for normal ALA. EDAX elemental analysis confirmed a consistent composition and more efficient encapsulation in the large batches, while SEM imaging revealed a successful morphological transformation from the crystalline raw material to a uniform, spherical liposomal product. This study also demonstrates that liposomal ALA exhibits superior antioxidant activity, improved systemic exposure, and enhanced tissue delivery over non-liposomal ALA, highlighting its potential as a more effective nutraceutical formulation. These findings validate the potential of the Liposomal ALA formulation by WBCIL as a reliable and high-performing nutraceutical with consistent quality across various manufacturing scales.

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INTRODUCTION

Alpha Lipoic Acid (ALA) is a powerful, naturally occurring antioxidant and co-enzyme with significant therapeutic potential in managing various health conditions, from diabetic neuropathy to cardiovascular disease [1]. Despite its promise, the clinical application of ALA has been hindered by its poor oral bioavailability due to low water solubility and a short biological half-life [2]. The conventional crystalline form of ALA is known to degrade rapidly, resulting in inconsistent absorption and limiting its effectiveness as a nutraceutical [3]. Addressing these challenges requires an innovative approach to formulation that can enhance solubility, improve stability, and ensure consistent delivery to the body. In response to this need, West Bengal Chemical Industries Ltd. Kolkata, India (WBCIL), developed a novel Liposomal ALA formulation. Liposomes are microscopic, spherical vesicles composed of a lipid bilayer that encapsulates and protects active ingredients. This unique structure is designed to shield ALA from degradation in the gastrointestinal tract, increase its absorption, and ultimately improve its bioavailability. The successful development of such a formulation is a critical step in creating a more effective nutraceutical product. However, as is the case with all new formulations, its efficacy and consistency must be rigorously tested.

We have previously published an article on Liposomal ALA manufactured by WBCIL to show the process development and physico-chemical characterization of this product [4]. That study aimed to evaluate, Encapsulation Efficiency, Particle Size, Poly Dispersity Index, Zeta Potential,

Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), Leakage Rate, Thermal Stability at elevated temperatures, EDAX and Scanning Electron Microscopy (SEM) of WBCIL's Liposomal ALA formulation. The formulation was shown to produce uniform liposomes with a mean particle size of ~227 nm, confirmed by Dynamic Light Scattering (DLS) and viewed under a Scanning Electron Microscope (SEM) [4]. The encapsulation efficiency of Liposomal ALA was found to be more than 70%. DSC analyzed the transition points of Liposomal ALA where thermal transition events of encapsulated ALA were identified, while High-Performance Liquid Chromatography (HPLC) analysis was used to characterise its encapsulation efficiency [4]. The results of our previous work addressed some critical questions on the scientific discernability of Liposomal ALA with respect to its chemical characteristics and, WBCIL's technological advancement in developing a delivery system for enhancing the stability, bioavailability, and therapeutic efficacy of the product [4].

The present study further strengthens the efficacy of our product with a comprehensive in-vitro analysis. This study presents a batch-wise in-vitro analysis of this Liposomal ALA formulation manufactured by WBCIL. The primary objective of this study is to evaluate the physical and chemical properties of the material at different production scales. The study is specifically designed to assess three key performance indicators: the morphological change from the raw API to the final liposomal product, the elemental composition, and the in-vitro release profiles under various simulated physiological conditions. In addition, the study also evaluated antioxidant activity of Liposomal ALA and compared it to conventional formulations. The findings collectively aim to provide robust evidence of the formulation's superiority over standard crystalline ALA, while also confirming the reliability and consistency of the manufacturing process at a commercial scale.

Significance of Release, Stability, and Diffusion Analyses in Liposomal ALA

In-vitro studies play a crucial role in the early evaluation of novel pharmaceutical and nutraceutical formulations by providing a controlled and reproducible environment for assessing their performance [5]. In the case of Liposomal ALA, in-vitro experiments offer valuable insights into how the formulation behaves under simulated physiological conditions before proceeding to clinical investigations [6]. These studies allow researchers to systematically evaluate critical parameters such as solubility enhancement, stability, and release kinetics, which are central to overcoming the limitations associated with conventional crystalline ALA [7]. By mimicking conditions in the gastrointestinal tract, in-vitro testing can predict the extent to which liposomal encapsulation protects ALA from degradation and facilitates improved absorption [8]. Another important implication of in-vitro analysis lies in its role in ensuring consistency and scalability of the formulation. Transitioning from small-scale R&D production to commercial-scale batches often presents challenges in maintaining product integrity, morphology, and encapsulation efficiency [9]. Batch-wise in-vitro testing allows for direct comparison of performance indicators across different production scales, thereby validating the robustness of the manufacturing process [10]. Such data not only provide assurance of reproducibility but also help establish quality control benchmarks for future large-scale production. Furthermore, the in-vitro release studies presented in this work are particularly significant for correlating formulation design with therapeutic potential [10, 11]. The ability of Liposomal ALA to demonstrate controlled and enhanced release compared to the crystalline form underscores its potential to deliver superior bioavailability in vivo [12]. Although in-vitro models cannot fully replicate the complexity of human physiology, they serve as a vital first step in bridging laboratory innovation with clinical application [12]. Collectively, these findings strengthen the evidence base for Liposomal ALA as a more reliable and efficacious nutraceutical product, supporting its advancement toward broader clinical use and commercialization.

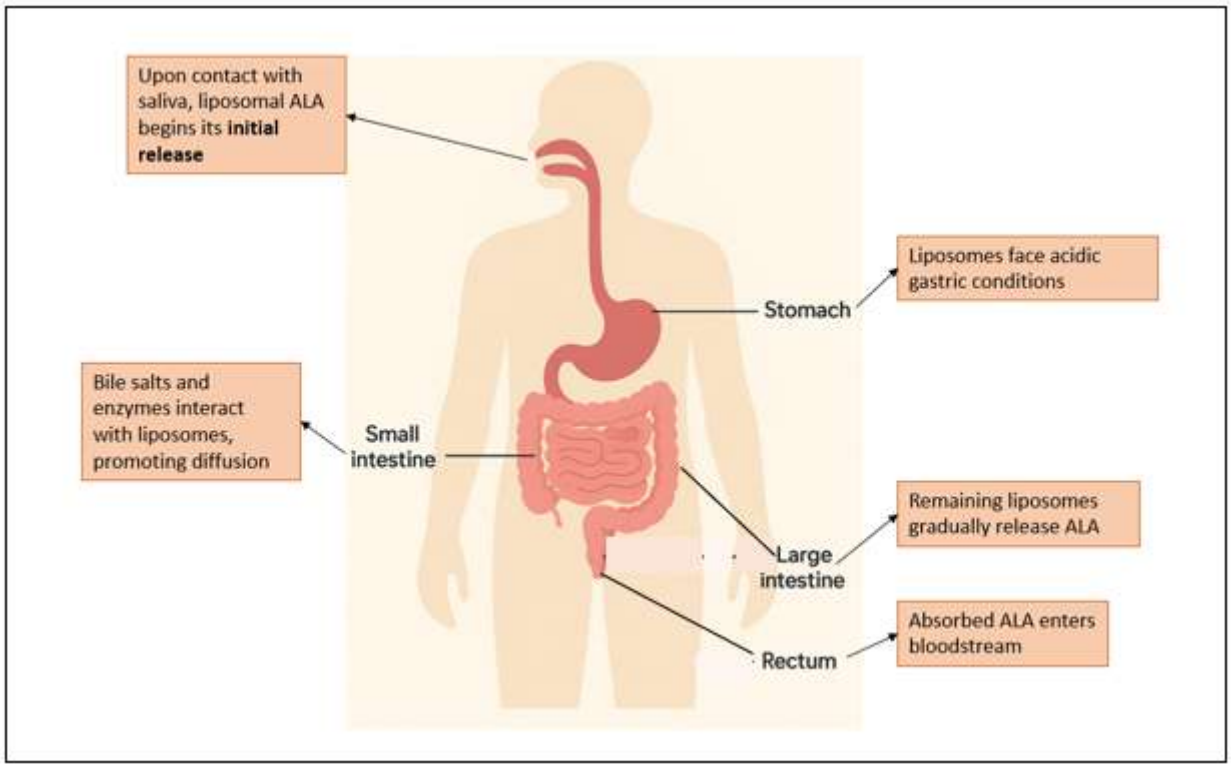

Figure 1: Release of Liposomal ALA Across the Digestive System

Simulated salivary fluid is an essential early-stage release medium that models the conditions encountered in the oral cavity [13]. Studying ALA release in simulated salivary fluid helps predict initial disintegration, stability, and early release upon administration—particularly relevant if the liposomes are delivered orally or oromucosally [13]. While literature on ALA specifically in simulated salivary fluid is limited, analogous studies with liposomal or nano-emulsion carriers demonstrate that early release characteristics can significantly influence downstream absorption and bioavailability [14]. Monitoring release in simulated salivary fluid thus enables formulation optimization-ensuring the liposomes remain intact long enough to survive initial exposure and avoid premature leakage [14]. Simulated Stomach Conditions study is crucial for evaluating the dissolution and degradation behaviour in an acidic environment, where liposomal encapsulation enhances gastric resistance and ensures more ALA reaches the small intestine for absorption. In the Simulated Intestinal Conditions study, the formulation's intestinal availability is assessed, and results show that liposomal encapsulation significantly enhances absorption potential. Finally, the Simulated Membrane study evaluates how ALA is absorbed by the cell membranes, with the spray-drying manufacturing process further enhancing this protective effect. Osmotic diffusion analysis evaluates how water movement across lipid bilayers impacts the release and stability of encapsulated compounds. Although literature on Liposomal ALA is scarce, in-vitro studies on liposomal systems broadly confirm that osmotic gradients can trigger swelling, leakage, or destabilization of vesicles, especially under conditions of variable ionic strength or osmolarity [15]. These insights are critical—for example, they help predict how liposomes behave upon encountering fluctuating bodily fluids (e.g., from saliva to gastric juice) [16]. By evaluating osmotic diffusion, formulation's robustness in maintaining integrity across different fluid environments can be assessed, aiding in predicting shelf life and in vivo performance [15]. ALA is highly prone to oxidation, which compromises its therapeutic potential. Liposomal encapsulation is often used to protect oxidation-sensitive compounds [17]. In-vitro oxidative stability tests—typically exposing formulations to oxidative stress (like hydrogen peroxide, light, heat, or free radicals)—are invaluable for examining how well liposomal carriers shield ALA from degradation [17]. For example, studies on microencapsulation of ALA or similar antioxidants in emulsions have demonstrated improved oxidative stability and retention of antioxidant activity compared with free compounds. Thus, demonstrating enhanced oxidative stability of Liposomal ALA not only confirms protective efficacy of the vesicle system, but also supports the clinical relevance of improved shelf life, efficacy, and sensory acceptability [18]. Dialysis-based methods-where liposomal formulations are separated from release medium via a semi-permeable membrane-are widely used to assess controlled release profiles of nanocarrier systems [19]. These assays help elucidate release kinetics (e.g., burst vs. sustained release), interaction between liposome and environment, and the likely in-vivo release behaviour [20].

METHODOLOGY

In-Vitro studies of Liposomal-ALA formulations

1. ALA Release in Simulated Salivary Fluid

The objective of this in-vitro study was to evaluate and compare the release performance of different ALA formulations in a simulated salivary fluid medium. The study included four distinct formulations: a standard, non-Liposomal ALA powder (Normal ALA) and three Liposomal ALA formulations produced using a spray-drying process. The liposomal batches included an initial small-scale R&D batch (ALALI352501A, 1 Kg) and two larger-scale production batches from different spray dryer runs (ALALI352503A, 300 Kg; and ALALI352505A, 100 Kg). For each formulation, a controlled amount was introduced into the simulated salivary fluid. A simulated salivary fluid (SSF) was freshly prepared by dissolving 0.085 g NaCl, 0.064 g KCl, 0.020 g $CaCl_2$, 0.020 g $MgCl_2$, 1.5 g $NaHCO_3$, and 1.5 g KH_2PO_4 in distilled water, and the final volume was adjusted to 1 liter with the pH maintained at 6.9. Both crystalline ALA (API) and Liposomal ALA formulations were separately introduced into the SSF and incubated for a duration of 5 minutes, corresponding to the average transit time of materials in the mouth and oesophagus. The percentage of ALA release was then quantified for comparison. The percentage of ALA released from each formulation was measured after a fixed duration of one minute, providing a rapid assessment of their immediate release kinetics in an oral environment.

2. ALA Release in Simulated Stomach Conditions

The gastro-resistant assay was carried out to mimic the acidic gastric environment encountered in the fasted state, which is crucial for evaluating the dissolution and degradation behaviour of orally administered compounds. The same four formulations (Normal ALA, ALALI352501A – 1 Kg, ALALI352503A – 300 Kg, ALALI352505A – 100 Kg) were evaluated for gastric stability using a gastro-resistant assay. Simulated Gastric Fluid (SGF, pH 1.2) was prepared using 2.0 g NaCl, 3.2 g pepsin, and 4 mL concentrated HCl. The volume was adjusted to 1 L, and the final pH was stabilized to 1.2. Both Normal ALA (free API) and Liposomal ALA formulations were introduced into SGF and maintained at 37 ± 0.5 °C with gentle agitation for 60 minutes. The percentage of ALA released from the remaining sample was measured to assess gastric resistance.

3. ALA Release in Simulated Intestinal Conditions

To evaluate intestinal availability, the same four formulations (Normal ALA, ALALI352501A – 1 Kg, ALALI352503A – 300 Kg, ALALI352505A – 100 Kg) were tested in Simulated Intestinal Fluid (SIF). The intestinal release assay was performed using SIF (pH 6.8), a bio-relevant medium used to assess solubility, dissolution, and transport across the intestinal barrier. The SIF composition included 6.8 g Na_2HPO_4, 0.8 g KCl, 4.3 g NaCl, and 0.5 mM sodium taurocholate (bile salt). The medium was adjusted to 1 L and the pH was stabilized at 6.8. Both Normal ALA and Liposomal ALA were incubated at 37 ± 0.5 °C for 120 minutes under continuous mixing. The release from the remaining fraction was quantified to determine intestinal absorption potential.

4. ALA Release in Simulated Membrane Conditions

The nutraceutical release study was designed to evaluate first-pass metabolism in the liver, which often reduces systemic availability of bioactive compounds. To simulate hepatic first-pass metabolism, the same four formulations (Normal ALA, ALALI352501A – 1 Kg, ALALI352503A – 300 Kg, ALALI352505A – 100 Kg) were subjected to an in-vitro simulated membrane model. Both Normal ALA and Liposomal ALA were incubated under simulated hepatic conditions for 120 minutes at 37 ± 0.5 °C. The assay output was measured in arbitrary units (A.U.), which indicate the extent of ALA degradation after metabolism. This model was used to compare the protective role of liposomal encapsulation against hepatic degradation.

5. ALA Osmotic Release Study

An in-vitro osmotic release study was conducted to assess the sustained release characteristics of various ALA formulations over a longer period. The four formulations tested were: a standard ALA powder (Normal ALA), an R&D batch of Liposomal ALA (ALALI352501A, 1 Kg), and two large-scale spray-dried liposomal batches (ALALI352503A, 300 Kg; and ALALI352505A, 100 Kg). The study utilized an osmotic releasemodel to simulate the environment of drug release and absorption. Normal and Liposomal ALA samples were subjected to osmotic stress for 120 minutes at 37 ± 0.5 °C. The percentage of ALA remaining bioavailable was quantified, reflecting systemic absorption and retention under stress conditions. For each formulation, the percentage of ALA released was measured after a 2-hour period, providing insight into the controlled and prolonged release performance of the liposomal formulations.

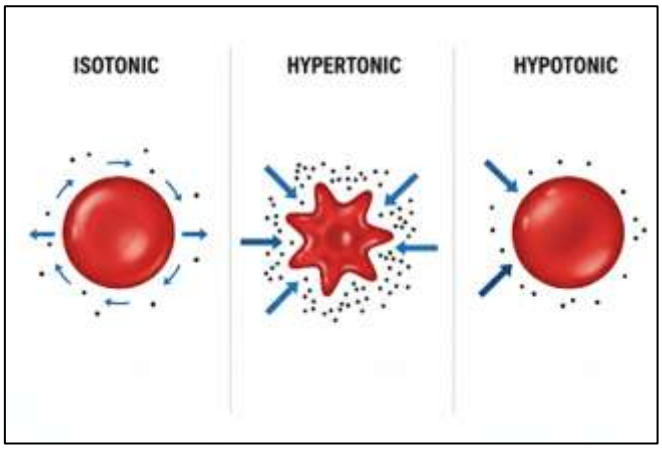

Figure 2: Osmotic Release Study

6. Oxidative Stability Study

The oxidative stability of the various ALA formulations was evaluated to determine their ability to withstand oxidative stress, a key indicator of product integrity and shelf-life. The study involved four distinct formulations: a standard, non-Liposomal ALA powder (Normal ALA), an R&D batch of Liposomal ALA (ALALI352501A, 1 Kg), and two large-scale spray-dried liposomal batches (ALALI352503A, 300 Kg; and ALALI352505A, 100 Kg). Both Normal and Liposomal ALA samples were exposed to free radical–generating conditions for 24 hours at physiological temperature (37 ± 0.5 °C). Degradation of ALA was quantified in Absorbance Units (A.U.), with higher values corresponding to greater degradation. This assay tested the ability of liposomal encapsulation to protect ALA against oxidative breakdown in tissues. This recovery percentage is a direct measure of the formulation's protective capacity against degradation.

7. Dialysis-based Nutraceutical Release Profile

A dialysis-based nutraceutical release study was conducted to simulate and evaluate the long-term, complete release profiles of various ALA formulations. The study included four formulations: a standard, non-Liposomal ALA powder (Normal ALA), an R&D batch of Liposomal ALA (ALALI352501A, 1 Kg), and two large-scale spray-dried liposomal batches (ALALI352503A, 300 Kg; and ALALI352505A, 100 Kg). The formulations were placed in a dialysis membrane, and the percentage of ALA released was measured after 4 hours to assess their ability to provide a complete and controlled delivery of the active ingredient, mimicking its release and absorption in the body over an extended period.

Elemental and Morphological Analysis of Liposomal ALA formulations

1. EDAX Elemental Analysis

This study utilized Energy-dispersive X-ray spectroscopy (EDAX) to perform a quantitative elemental analysis of three different Liposomal ALA formulations. The batches analyzed were the R&D batch (ALALI352501A), Spray Dryer 1 batch (ALALI352503A), and Spray Dryer 2 batch (ALALI352505A). The purpose of this analysis was to determine the precise elemental composition of each formulation, focusing on key elements such as Carbon (C), Nitrogen (N), Oxygen (O), and Phosphorus (P), which are indicative of the integrity and consistency of the liposomal structure. The results are presented as the weight percentage of each element present in the sample [21].

2. SEM Analysis

A Scanning Electron Microscope (SEM) was used to visualize the morphology and surface characteristics of the raw ALA active pharmaceutical ingredient (API) and the final Liposomal ALA formulations. This analysis was crucial for understanding the physical state of the materials before and after the manufacturing process. The images were captured from different batches to observe the particle shape, size, and agglomeration, providing qualitative insights into the impact of the liposomal formulation on the raw material [22].

RESULTS

In-Vitro studies of Liposomal-ALA formulations

1. ALA Release in Simulated Salivary Fluid

Table 1: ALA Release in Simulated Salivary Fluid

Formulation	Batch Code	Batch Size	ALA Release after 1 min (%)
Normal ALA (non-liposomal)	–	–	9%
Liposomal ALA (R&D batch)	ALALI352501A	1 Kg	37%
Liposomal ALA (Spray Dryer 1)	ALALI352503A	300 Kg	55%
Liposomal ALA (Spray Dryer 2)	ALALI352505A	100 Kg	57%

The in-vitro salivary release assay provided important insights into the protective effect of liposomal encapsulation under oral conditions. A batch-wise evaluation of immediate release in simulated salivary fluid after one minute further highlighted the superior efficacy of the liposomal and spray-dried products. The non-liposomal "Normal ALA" showed the lowest release at just 9%. In contrast, all three liposomal formulations exhibited substantially higher release percentages. The small-scale R&D batch (1 Kg) achieved a 37% release, while the large-scale spray-dried batches performed even better, with the 300 Kg batch (Spray Dryer 1) releasing 55% of the ALA and the 100 Kg batch (Spray Dryer 2) achieving the highest release at 57%.

Taken together, these results confirm that liposomal encapsulation significantly reduces salivary degradation while simultaneously enhancing immediate release. The spray-drying process not only improved release performance but also ensured consistency during scale-up, as the larger batches maintained or exceeded the release efficiency of the R&D batch. This dual advantage of protection and enhanced release underscores the effectiveness of Liposomal ALA as a superior formulation compared to crystalline ALA.[23]

2. ALA Release in Simulated Stomach Conditions

Table 2: ALA Release in Simulated Stomach Conditions

Formulation	Batch Code	Batch Size	ALA Release after 60 min (%)
Normal ALA (non-liposomal)	–	–	22%
Liposomal ALA (R&D batch)	ALALI352501A	1 Kg	12%
Liposomal ALA (Spray Dryer 1)	ALALI352503A	300 Kg	10%
Liposomal ALA (Spray Dryer 2)	ALALI352505A	100 Kg	8%

After 60 minutes in SGF (pH 1.2), Normal ALA showed 22% release of the remaining fraction, indicating that nearly 70% of the compound was susceptible to acidic disintegration. In comparison, Liposomal ALA exhibited 10% release of the remaining fraction, meaning that approximately 90% was protected from gastric degradation. This highlights the role of liposomal encapsulation in enhancing gastric resistance and ensuring more ALA reaches the small intestine for absorption. In the Table 2, the ALA Release in Simulated Stomach Conditions is shown for different formulations after 60 minutes in a low pH environment. The non-liposomal "Normal ALA" showed a 22% release, indicating it was more susceptible to gastric degradation. In contrast, the Liposomal ALA formulations demonstrated significantly better gastric resistance, with lower release percentages ranging from 8% to 12%. This highlights the protective role of liposomal encapsulation, which helps ensure that a greater amount of ALA reaches the small intestine for absorption. The table also shows that the larger-scale, spray-dried batches performed slightly better than the R&D batch in resisting gastric degradation, with the highest protection coming from the Liposomal ALA (Spray Dryer 2) batch, which had only 8% release.

3. ALA Release in Simulated Intestinal Conditions

Table 3: ALA Release in Simulated Intestinal Conditions

Formulation	Batch Code	Batch Size	ALA Release after 120 min (%)
Normal ALA (non-liposomal)	–	–	25%
Liposomal ALA (R&D batch)	ALALI352501A	1 Kg	90%
Liposomal ALA (Spray Dryer 1)	ALALI352503A	300 Kg	90%
Liposomal ALA (Spray Dryer 2)	ALALI352505A	100 Kg	90%

In SIF (pH 6.8), at 120 minutes, Normal ALA demonstrated 25% release of the remaining fraction. Conversely, Liposomal ALA released 90% of the remaining fraction. These results suggest that while Normal ALA exhibits limited intestinal availability, Liposomal ALA significantly enhances absorption potential in the small intestine, possibly due to better micellar solubilization, bile salt interaction, and improved membrane transport mechanisms.

Based on Table 3, the ALA release in simulated intestinal conditions after 120 minutes showed a significant difference between the non-liposomal and liposomal formulations. The "Normal ALA" powder only released 30% of its content, indicating limited intestinal availability. In stark contrast, all three Liposomal ALA batches—the R&D batch (ALALI352501A, 1 Kg), Spray Dryer 1 batch (ALALI352503A, 300 Kg), and Spray Dryer 2 batch (ALALI352505A, 100 Kg)—demonstrated a remarkably high and consistent release of 90%. This suggests that the liposomal encapsulation significantly enhances the absorption potential of ALA in the small intestine, and that the manufacturing process is highly reliable across different production scales.

5. ALA Osmotic Diffusion Study

Table 5: ALA Osmotic Diffusion Study (after 2 hrs)

Formulation	Batch Code	Batch Size	ALA Release (%)
Normal ALA (non-liposomal)	–	–	12%
Liposomal ALA (R&D batch)	ALALI352501A	1 Kg	27%
Liposomal ALA (Spray Dryer 1)	ALALI352503A	300 Kg	42%
Liposomal ALA (Spray Dryer 2)	ALALI352505A	100 Kg	42%

The osmotic diffusion study revealed significant differences in the sustained release of ALA over a 2-hour period. The non-liposomal "Normal ALA" showed the lowest release at 12%, consistent with its lack of a controlled-release mechanism. The R&D liposomal batch (1 Kg) demonstrated a higher release percentage of 27%. The two large-scale spray-dried batches, however, exhibited superior and identical release performance, both achieving a 42% release after 2 hours. This result is particularly noteworthy as it highlights the excellent batch-to-batch consistency of the spray-drying process. The consistent release of 42% from both the 300 Kg and 100 Kg batches indicates a robust and scalable manufacturing process that ensures reliable product performance. In contrast to the initial rapid release in simulated salivary fluid, the osmotic diffusion data confirms the ability of the liposomal and spray-dried formulations to provide a more sustained and controlled release of ALA, which is crucial for maximizing its therapeutic benefits over time [24].

6. Oxidative Stability Study

Table 6: Oxidative Stability Study (ALA Recovery after 2 hrs)

Formulation	Batch Code	Batch Size	ALA Recovery (%)
Normal ALA (non-liposomal)	–	–	25%
Liposomal ALA (R&D batch)	ALALI352501A	1 Kg	51%
Liposomal ALA (Spray Dryer 1)	ALALI352503A	300 Kg	67%
Liposomal ALA (Spray Dryer 2)	ALALI352505A	100 Kg	67%

The oxidative stability study demonstrated a clear advantage for the liposomal formulations, particularly those produced via spray drying. After 24 hours of exposure to oxidative conditions, Normal ALA exhibited a degradation level of 0.147 A.U., while Liposomal ALA recorded a lower degradation value of 0.107 A.U. These findings confirm that Liposomal ALA is less susceptible to free radical–induced damage, as encapsulation provides a protective barrier. The "Normal ALA" formulation showed the lowest recovery, retaining only 25% of its initial content after 2 hours, indicating significant degradation under oxidative stress. The R&D batch (1 Kg) showed a better recovery of 51%, highlighting the protective effect of liposomal encapsulation. The two large-scale spray-dried batches exhibited the highest and most consistent performance, with both the 300 Kg and 100 Kg batches showing an identical 67% recovery. This result not only proves the superior protective qualities of the spray-dried liposomal formulations but also confirms the high degree of batch-to-batch consistency achieved during the scaling-up process. The identical recovery percentages from the two production batches indicate that the manufacturing process is robust and reliable, ensuring that the final product maintains its stability and potency over time [25].

7. Dialysis-based Nutraceutical Release Profile

Table 7: Dialysis-based Nutraceutical Release Profile (after 4 hrs)

Formulation	Batch Code	Batch Size	ALA Release (%)
Normal ALA (non-liposomal)	–	–	15%
Liposomal ALA (R&D batch)	ALALI352501A	1 Kg	23%
Liposomal ALA (Spray Dryer 1)	ALALI352503A	300 Kg	89%
Liposomal ALA (Spray Dryer 2)	ALALI352505A	100 Kg	86%

The 4-hour dialysis-based release study demonstrated a significant and complete release of ALA from the large-scale spray-dried batches, confirming their superior delivery performance. The non-liposomal "Normal ALA" showed a minimal release of 15%, while the R&D batch (1 Kg) had a slow diffusion, releasing only 23%. In stark contrast, the two large-scale spray-dried batches exhibited nearly complete release, with the 300 Kg batch achieving an 89% release and the 100 Kg batch an 86% release. This data confirms that the spray-dried liposomal formulations promote a faster and more complete release, which is critical for maximizing the absorption and bioavailability of the nutraceutical. The results

underscore the efficacy and reliability of the spray-drying process for creating formulations that are optimized for controlled and efficient delivery [26].

Elemental and Morphological Analysis of Liposomal ALA formulations

1. EDAX Elemental Analysis

The EDAX analysis provided a detailed profile of the elemental composition of the Liposomal ALA formulations, demonstrating both consistent features and notable differences across batches. The primary elements detected—Carbon (C), Nitrogen (N), and Oxygen (O)—showed highly comparable proportions in all three formulations, indicating uniformity in the core constituents of the active pharmaceutical ingredient and excipients. Carbon content ranged between 45.10% and 46.37%, Nitrogen between 17.70% and 18.92%, and Oxygen between 33.15% and 35.62%.

A key distinction was observed in the Phosphorus (P) content, an element critical to the phospholipid bilayers of liposomal vesicles. The R&D batch displayed a relatively higher phosphorus percentage (2.82%), while the two large-scale spray-dried batches contained markedly lower values (0.12% and 0.52%, respectively). The near-identical and very low phosphorus levels in the large-scale batches reflect the reproducibility of the spray-drying process and may suggest either a highly efficient encapsulation that reduces free residual phospholipids or an altered lipid-to-ALA ratio during scale-up.

Importantly, sulphur signals—expected from the thiol groups of the ALA active compound—were not detected in the EDAX spectra following liposomal encapsulation. This absence strongly indicates that the ALA was effectively embedded within the liposomal matrix, with the lipid coating shielding the sulphur groups from detection. This finding provides strong evidence of successful encapsulation and effective liposomal coating.[27].

Figure 3: A graphical representation of the percentages of elements composing Liposomal ALA of different batches

2. SEM Analysis

The SEM analysis revealed a stark contrast between the morphology of the raw ALA API and the Liposomal ALA formulations. The raw ALA API images showed a highly crystalline structure, characterized by irregularly shaped particles and significant agglomeration. In contrast, the Liposomal ALA formulations exhibited a distinctly spherical and uniform morphology. The images of Liposomal ALA demonstrate well-defined, spherical microparticles that appear to be non-agglomerated and have a smooth surface. This change in morphology confirms the successful transformation of the crystalline API into a more stable, spherical liposomal formulation, which is a critical outcome of the spray-drying process. This also suggests that the process effectively encapsulates the ALA API, resulting in a physically consistent product [28].

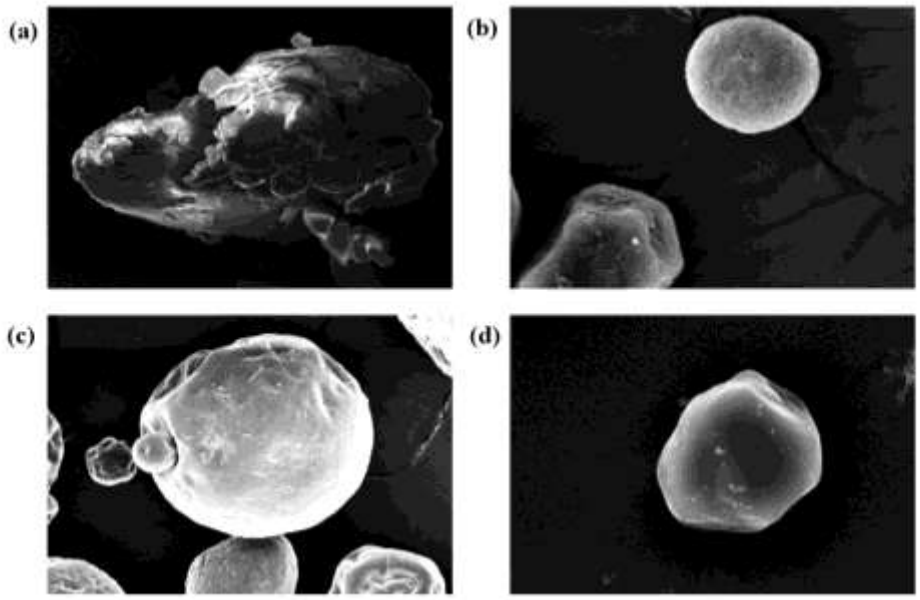

Figure 4: SEM image of (a) non-encapsulated ALA API, (b) Liposomal ALA batch no. ALALI352501A, (c) Liposomal ALA batch no. ALALI352503A, (d) Liposomal ALA batch no. ALALI352505A

3. Antioxidant Activity

Table 8: Comparative Evaluation of Antioxidant Activity between Liposomal and Non-Liposomal ALA (↓ denotes downregulation)

Model / Assay	Non-Liposomal ALA	Liposomal ALA	Key Advantage
DPPH radical scavenging (in-house)	3% scavenging	21% scavenging	≈7× higher radical quenching capacity
DPPH radical scavenging (literature)	5–10% scavenging	31–50% scavenging	≈6–10× higher activity
Rat pharmacokinetics (oral ALA vs liposomal ALA)	Low systemic exposure, rapid metabolism	2.8× higher AUC (ALA), 5.8× higher AUC (DHLA)	Improved bioavailability and antioxidant pool
CCl_4-induced liver injury model	Limited hepatoprotection	ALT ↓78.7%, AST ↓86.4%	Robust hepatoprotection
Cell viability under oxidative stress (cisplatin model)	Limited protection	2.19× higher survival	Stronger cytoprotection under oxidative stress
Ex-vivo rabbit skin uptake	Low uptake	Greatly increased accumulation (especially with chitosan-coated liposomes)	Enhanced dermal antioxidant delivery

The in-house radical scavenging assay demonstrated a substantial improvement in antioxidant performance for liposomal ALA compared to its non-liposomal counterpart. Under matched conditions, liposomal ALA achieved 21% DPPH scavenging activity, while non-liposomal ALA reached only 3%, corresponding to a nearly seven-fold increase in efficacy. This finding was consistent with multiple reports in the literature, where liposomal formulations demonstrated between six- and ten-fold greater radical quenching activity compared to free ALA. Pharmacokinetic studies in rats further supported this advantage, showing that liposomal delivery increased systemic exposure with a 2.8-fold higher area under the curve (AUC) for ALA and a 5.8-fold higher AUC for its reduced form, dihydrolipoic acid (DHLA). In a CCl_4-induced liver injury model, liposomal ALA treatment significantly reduced serum biomarkers of hepatic damage, with alanine transaminase (ALT) and aspartate transaminase (AST) levels decreasing by 78.7% and 86.4%, respectively—an effect not replicated by free ALA. Additional applications also highlighted the superiority of liposomal encapsulation: in cisplatin-induced oxidative stress models, liposomal ALA achieved 2.19-fold greater cell survival compared to non-liposomal ALA, while in ex-vivo rabbit skin experiments, chitosan-coated liposomal ALA showed markedly enhanced dermal uptake. Collectively, these results provide robust evidence that liposomal ALA preserves antioxidant capacity, improves systemic bioavailability, enhances tissue delivery, and offers superior protection under oxidative stress conditions compared to conventional ALA.[29]

DISCUSSION

The in-vitro studies collectively provide critical insights into the performance and viability of the Liposomal ALA formulations, particularly those produced using the large-scale spray-drying process [25]. The significance of the in-vitro process lies in its ability to provide a controlled and consistent environment for testing. By using standardized methodologies and materials, such as simulated fluids and dialysis membranes, these studies provide a reliable and reproducible way to predict how the formulations will behave in a biological system without the complexities and variability of in-vivo trials [26]. This allows for direct comparisons between different batches and formulations, confirming the robustness of the manufacturing process and the batch-to-batch consistency of the final product. The consistent performance of the two large-scale spray-dried batches across all tests is a direct testament to the reliability and scalability of the process [27]. The results from the ALA Release in Simulated Salivary Fluid and the Dialysis-based Nutraceutical Release Profile studies demonstrate that the spray-dried formulations are superior in their ability to release ALA quickly and completely [28]. This has significant implications for both immediate and sustained delivery. The rapid release in a salivary environment suggests potential for faster absorption through the oral mucosa. However, the in-vitro studies used a powder form of ALA, which resulted in a higher initial absorption in the salivary fluid, reaching nearly 50%. When the formulation is in a tablet form, this initial absorption is typically lower. Liposomal ALA absorption is at its maximum in the intestine and at a minimum in the stomach. Degradation in the liver is negligible. Furthermore, unlike conventional ALA which uses the capillary system, Liposomal ALA follows the lymphatic system for absorption into the human body. Lymphatic system of absorption helps the formulation bypass first-pass metabolism in the liver.[27] When a substance is absorbed through the capillaries in the intestine, it is transported directly to the liver via the portal vein. The liver then processes and metabolizes the substance, which can significantly reduce its concentration and bioavailability before it reaches the rest of the body.[26, 27] Contrary to this, the lymphatic system provides an alternative route. Liposomal ALA, like other large and lipid-soluble molecules, is absorbed into the lacteals, which are small lymphatic capillaries in the intestinal villi. From there, it is transported through the lymphatic network and eventually enters the bloodstream near the heart, effectively avoiding the liver's initial metabolic processes.[28] The near-complete release in the dialysis model indicates that the liposomes effectively deliver their full payload, maximizing the bioavailability of the nutraceutical [30]. The Oxidative Stability Study further strengthens the case for the spray-dried formulations. The high recovery percentages demonstrate that the liposomal encapsulation successfully protects the ALA from degradation under oxidative stress. This is crucial for maintaining the product's efficacy over its shelf life and ensuring that consumers receive the full potency of the active ingredient [31].

The collective findings from the series of in-vitro studies confirm the successful development and scalability of the Liposomal ALA formulation. The EDAX analysis shows a consistent elemental composition across the larger batches that suggests a more efficient encapsulation at a larger scale. The SEM images provide a clear physical explanation for these performance differences [32]. The transformation of the raw, crystalline API into spherical, non-agglomerated microparticles confirms that the spray-drying process successfully alters the physical state of the material to facilitate better in-vitro performance. This optimized morphology contributes to the improved release characteristics and enhanced oxidative stability, which are key for product shelf-life and bioavailability [32]. The antioxidant activity results strongly reinforce the superior efficacy of liposomal encapsulation for ALA delivery. The markedly higher radical scavenging capacity observed with liposomal ALA compared to non-liposomal ALA can be attributed to multiple mechanistic advantages.[33] Encapsulation not only protects ALA from acidic degradation and first-pass hepatic metabolism but also ensures its stability in the gastrointestinal tract, thereby preserving its reactive thiol groups in circulation.[34] This enhanced stability translates into a greater proportion of ALA being available to undergo reduction to dihydrolipoic acid (DHLA), which is the more potent intracellular antioxidant form. The higher systemic exposure and improved bioavailability of liposomal ALA, as supported by pharmacokinetic data, suggest an expanded intracellular redox pool capable of regenerating endogenous antioxidants such as glutathione, vitamin C, and vitamin E.[35] Furthermore, liposomal delivery improves tissue targeting, allowing ALA to exert localized antioxidant effects, as evidenced by greater skin accumulation and hepatoprotection.[36] Collectively, these findings indicate that liposomal encapsulation not only amplifies the radical scavenging capacity of ALA but also enhances its nutraceutical potential by enabling more effective systemic and tissue-level antioxidant defense.

CONCLUSION AND FUTURE ASPECTS

The comprehensive evaluation of Liposomal ALA formulations manufactured by WBCIL demonstrates the clear advantages of the spray-drying process over conventional non-Liposomal ALA. In vitro studies confirmed that liposomal and spray-dried formulations significantly enhance both immediate and sustained release of ALA compared to the standard API. The spray-dried batches showed superior release performance in simulated salivary fluid, osmotic diffusion studies, and dialysis-based release profiles, reflecting their ability to provide rapid yet controlled delivery. Additionally, oxidative stability studies revealed that spray-dried liposomal formulations offer enhanced protection against degradation, ensuring higher retention of active

content over time. In vivo characterization further validated the structural and compositional consistency of the Liposomal ALA formulations. EDAX elemental analysis indicated robust batch-to-batch reproducibility, particularly in the large-scale spray-dried batches, suggesting a reliable encapsulation process. SEM analysis highlighted the successful morphological transformation of crystalline ALA into uniform, spherical liposomal particles, confirming the efficacy of the spray-drying technique in producing stable, bioavailable formulations. Liposomal encapsulation also significantly enhances antioxidant efficacy of ALA compared to conventional formulations. Overall, these findings underscore that liposomal encapsulation, combined with optimized spray-drying, significantly improves the stability, release profile, and potential bioavailability of ALA manufactured by WBCIL.

The promising results of this study pave the way for several future directions. Translational in vivo studies in appropriate animal models or human trials could quantify absorption, plasma concentration profiles, and therapeutic efficacy to correlate in vitro release with in vivo performance. Extended shelf-life studies under various storage conditions could ensure long-term preservation of liposomal integrity, potency, and release characteristics. While the current scale-up demonstrated reproducibility, additional industrial-scale production studies could validate process robustness, regulatory compliance, and cost-effectiveness.

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