

# Preparation and Characterization of Liposomal N-Acetylcysteine

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## ABSTRACT

Liposomes are versatile lipid-based nanocarriers that can improve drug protection, delivery and release characteristics. This study aimed to develop and optimize a liposomal N-acetylcysteine (Lipo-NAC) formulation using a Box-Behnken design (BBD), with particle size and entrapment efficiency (EE) as the principal responses. Phospholipid, cholesterol and N-acetylcysteine concentrations were selected as independent formulation variables, and Design-Expert software was used for experimental design, modeling and numerical optimization. Liposomes were prepared by the thin-film hydration method using soy lecithin and cholesterol and were subsequently characterized by dynamic light scattering, zeta-potential analysis, transmission electron microscopy (TEM), UV spectrophotometry and short-term stability testing. The optimized formulation showed a mean particle size of  $65.8 \pm 2.05$  nm and a polydispersity index (PDI) of  $0.291 \pm 0.02$ . The measured zeta potential was  $-47.8$  mV, consistent with substantial electrostatic repulsion between vesicles. The manuscript dataset reports an optimized EE of  $72.5 \pm 0.36\%$ ; a separate calculation in the source document reports  $71.48\%$ , which requires final reconciliation with the underlying assay records before submission. TEM demonstrated predominantly spherical vesicular morphology. During 30 days of storage, EE was reported to remain at  $52\%$  under refrigeration at  $4^\circ\text{C}$ , whereas room-temperature storage showed a progressive decline. Overall, the findings support the feasibility of developing nanosized Lipo-NAC by thin-film hydration and response-surface optimization. Further work should establish validated analytical methods, long-term stability, release behavior and in vivo neuro-protective efficacy before therapeutic claims can be made.

**Keywords:** N-acetylcysteine, liposomes, Box-Behnken design, particle size, entrapment efficiency

## INTRODUCTION

N-acetylcysteine (NAC) is a thiol-containing compound widely recognized for its antioxidant activity and its role as a precursor for glutathione synthesis. Beyond its established clinical use as an antidote for acetaminophen toxicity, NAC has been investigated for potential applications in neurological disorders because of its antioxidant, anti-inflammatory and redox-modulating properties<sup>1-3</sup>. Reviews of NAC in neurological disease have described experimental evidence relevant to multiple sclerosis and other disorders in which oxidative stress and inflammatory signaling contribute to neuronal injury<sup>4</sup>.

Liposomal drug-delivery systems consist of phospholipid bilayer vesicles capable of incorporating hydrophilic compounds into the aqueous compartment and interacting with hydrophobic compounds within the lipid bilayer. Their physicochemical properties can be adjusted by changing lipid composition, cholesterol content, vesicle size, surface charge and

preparation conditions<sup>5-7</sup>. Such characteristics make liposomes attractive carriers for improving drug protection and controlling drug disposition.

Among the different preparation approaches, thin-film hydration is a widely used laboratory method in which lipids are dissolved in an organic solvent, the solvent is removed to form a dry lipid film, and the film is hydrated with an aqueous phase containing the drug or drug solution<sup>5,8</sup>. The resulting vesicles may subsequently be reduced in size by sonication or other size-reduction techniques. Because formulation variables can simultaneously affect several quality attributes, design-of-experiments approaches are useful for systematic optimization.

The Box-Behnken design is a response-surface methodology that allows simultaneous evaluation of multiple factors and responses while reducing the number of experimental runs compared with some full-factorial approaches<sup>9</sup>. Desirability-based optimization can then be used to identify a compromise

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formulation that meets predefined goals for multiple responses.

The present study therefore aimed to formulate Lipo-NAC using soy lecithin and cholesterol, optimize the formulation using a three-factor Box-Behnken design, and characterize the optimized formulation with respect to particle size, PDI, zeta potential, morphology and drug entrapment. Short-term stability was also examined under refrigerated and room-temperature conditions.

## MATERIALS AND METHODS

N-acetylcysteine, soy lecithin and cholesterol were used for formulation development. Chloroform was used as the organic solvent during lipid-film formation, and distilled water was used for hydration. The instrumentation utilized included a UV spectrophotometer, FTIR spectrophotometer, dynamic light-scattering/zeta-potential analyzer, probe sonicator, rotary evaporator and transmission electron microscope.

For preformulation characterization, FTIR data for pure NAC were obtained using the potassium bromide disc method. FTIR spectra of cholesterol, soy lecithin and the combined formulation components were also recorded for physicochemical characterization and assessment of chemical compatibility<sup>10-12</sup>.

A three-factor Box-Behnken design was generated using Design-Expert software (version 11). The independent variables were phospholipid concentration (A), cholesterol concentration (B) and NAC concentration (C). The dependent responses were particle size (PS) and entrapment efficiency (EE). Seventeen experimental runs were generated. Analysis of variance (ANOVA) was used to assess model significance and the contribution of formulation factors. Numerical optimization was performed using desirability-based constraints, with the goal of minimizing particle size while maximizing EE<sup>9</sup>.

Lipo-NAC was prepared by the thin-film hydration method. Soy lecithin and cholesterol were accurately weighed and dissolved in chloroform in a round-bottom flask. The organic phase was evaporated using a rotary evaporator at approximately 45-46°C for 15 min at 70 rpm under vacuum, producing a thin lipid film on the inner wall of the flask. NAC was dissolved

in 5 mL distilled water, and the lipid film was hydrated with 20 mL distilled water with rotation/stirring at room temperature. The resulting suspension was probe-sonicated for 30 min. The formulation was centrifuged at 36,000 rpm for 30 min to separate unencapsulated drug. The resulting liposomal preparation was lyophilized and stored at 2-8°C until analysis<sup>8,13</sup>.

Particle size and PDI were measured by dynamic light scattering with a 60-s measurement time and a scattering angle of 165°. Zeta potential was measured at 25°C using a 90° detection angle. Morphology was assessed by TEM. Entrapment efficiency was determined by measuring untrapped NAC in the post-centrifugation supernatant using UV spectrophotometry and calculating the difference between the total amount of drug initially added (W) and the amount detected in the supernatant (w) via the following equation:  $\text{Entrapment efficiency} = \left[ \frac{W-w}{W} \right] \times 100\%$

Optimized Lipo-NAC samples were sealed in 20-mL glass vials and stored for 30 days at 4°C or at room temperature (25±2°C; 60±5% relative humidity). Samples were examined at predetermined intervals (0, 10, 20 and 30 days) for drug entrapment using UV measurement at 205 nm for the stability assessment.

## RESULTS AND DISCUSSION

The Box-Behnken design generated 17 experimental runs, demonstrating substantial variation in particle size and EE across the formulation space. ANOVA evaluation of the model terms showed that phospholipid concentration (factor A) was significant for EE, while cholesterol concentration (factor B) was significant for particle size. The reported lack-of-fit p-values were >0.05 for both responses, indicating non-significant lack of fit. Response-surface and contour plots indicated that increasing phospholipid and cholesterol levels generally increased particle size. For EE, cholesterol exerted a positive influence, and its interaction with phospholipid levels suggested a combined influence on the lipid matrix.

The desirability approach identified an optimized formulation with a predicted particle size of 69.968 nm and a predicted EE of 76.575%. The experimentally observed values showed reasonable agreement with these predictions, as summarized in Table 1.

**Table 1: Experimental Validation of the Optimized Lipo-Nac Formulation**

| Sr. No. | RESPONSE              | PREDICTED | OBSERVED     |
|---------|-----------------------|-----------|--------------|
| 1       | Particle size         | 69.968 nm | 65.8±2.05 nm |
| 2       | Entrapment efficiency | 76.575%   | 72.5±0.36%   |

The optimized Lipo-NAC showed a mean particle size of 65.8±2.05 nm and a low PDI of 0.291±0.02, reflecting a narrow and uniform particle-size distribution. Nanometer-scale distribution is a critical quality attribute for liposomal delivery systems because it heavily dictates in vivo disposition<sup>14</sup>. The measured zeta potential was -47.8 mV, indicating a strongly negative surface charge. While the initial target range was noted as ±30 mV, magnitudes greater than 30 mV imply high physical stability due to substantial electrostatic vesicle-vesicle repulsion, though further long-term testing is needed to completely rule out slow aggregation over time<sup>14,15</sup>. TEM examinations verified that the structural morphology consisted of predominantly spherical vesicular networks.

The primary dataset reports an observed EE of 72.5±0.36%. However, a secondary data entry mentions an untrapped NAC quantity of 71,308 µg derived from an initial total of 250,000 µg. Applying the standard mathematical equation: 
$$EE = \left[ \frac{250,000 - 71,308}{250,000} \right] \times 100 = 71.48\%$$
 This yields 71.48%, revealing a slight internal discrepancy against the alternative 75.47% phrase noted in baseline laboratory reports.

Authors must confirm the verified calibration standards and batch assay sheets prior to journal routing.

Stability testing demonstrated that temperature plays an essential role in vesicle retention. Under refrigeration (4°C), the drug entrapment value stayed constant at 52% across the full 30-day timeline. Conversely, storage at room temperature (25±2°C) triggered an incremental leakage of the compound over the tracking intervals, as contextualized in Table 2.

**Table 2: Short-Term Stability Performance Evaluation Across 30 Days**

| Sr. No. | STORAGE CONDITION | DAY 0 | DAY 10 | DAY 20 | DAY 30 |
|---------|-------------------|-------|--------|--------|--------|
| 1       | 4°C               | 52%   | 52%    | 52%    | 52%    |
| 2       | 25±2°C            | 52%   | 49%    | 46%    | 41%    |

The apparent decrease from the freshly optimized experimental EE (72.5%) down to a baseline of 52% at Day 0 of the stability run indicates a potential change in sampling protocols, batch volumes, or tracking conditions (such as the shift from 254 nm to 205 nm analytical detection wavelengths). This structural variance highlights that refrigeration provides superior short-term protection, but complete long-term validation must be established to satisfy international criteria.

## CONCLUSION

A liposomal formulation of N-acetylcysteine was successfully developed using soy lecithin and cholesterol and optimized by Box-Behnken response-surface methodology. The optimized formulation exhibited a nanoscale particle size (65.8±2.05 nm), PDI of 0.291±0.02, and zeta potential of -47.8 mV, with predominantly spherical morphology by TEM. Short-term storage at 4°C showed better retention of entrapped drug than room-temperature storage. These findings support further development of Lipo-NAC as a candidate nanocarrier for studies of NAC delivery. However, the minor discrepancies in mathematical entrapment percentages and UV tracking wavelengths

must be finalized against the raw laboratory notebooks, and comprehensive evaluations of long-term kinetics, scaling mechanics, and in vivo efficacy remain necessary before clinical translations can progress.

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