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Development of Gallic Acid-loaded Liposomes to Combat Non-alcoholic Steatohepatitis

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Abstract

Background: This research explores the development of a novel herbal-targeted therapy leveraging Gallic Acid (GA), a polyphenolic phytoconstituent abundant in plants such as tea, grapes, and gallnuts, renowned for its potent anti-inflammatory, antioxidant, and lipid-modulating properties. Targeting the liver is of critical importance in Non-Alcoholic Steatohepatitis (NASH) therapy. A novel drug delivery approach, biocompatible and biodegradable vesicular carriers capable of improving drug stability, drug permeability, and targeted delivery to hepatic cells. **Aim:** To develop and optimise gallic acid-loaded liposomal formulations using thinfilm hydration and investigate the influence of formulation variables (lipid concentration, sonication time) on particle size and encapsulation of gallic acid liposomes using a Box–Behnken design, characterise their physicochemical properties and evaluate *in vitro* release kinetics with controlled Higuchi diffusion-controlled release for potential therapy of NASH. **Methods:** The study involves the formulation and optimisation of gallic acid-loaded liposomes using techniques such as thin-film hydration and the Box-Behnken design to achieve optimal particle size. **Results:** The optimised formulation exhibited an average particle size of approximately 2.7 µm with a drug encapsulation efficiency of 80% after a Fourier Transform Infrared Spectroscopy (FTIR) study of pure gallic acid. The Higuchi model showed the highest R^2 value (0.982); the drug release follows the Higuchi diffusion-controlled release mechanism. **Conclusion:** This research aims to prepare and evaluate a Gallic acid-loaded liposome formulation, perform physicochemical characterisation, and assess *in vitro* dissolution to ensure enhanced delivery and therapeutic performance

Major Findings: $Y=b_0 + 0.753A + 0.223C$; according to this equation, where y is particle size(µm), and b_0, the y-intercept, is 0.024, the optimised gallic acid-loaded drug indicates that lipid concentration and sonication time significantly influenced particle size.

Keywords: Encapsulation, Gallic Acid, *In Vitro* Dissolution, Non-alcoholic Steatohepatitis, Optimisation, Particle Size, Encapsulation

Abbreviations: GA: Gallic Acid; FTIR: Fourier Transform Infrared Spectroscopy; NASH: Non-alcoholic Steatohepatitis; NAFLD: Non-alcoholic Fatty Liver Disease; EPR: Enhanced Permeability and Retention.

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1. Introduction

Gallic Acid (GA), a natural polyphenol from plants like grapes and tea, shows promise in treating NASH, the aggressive form of Non-Alcoholic Fatty Liver Disease (NAFLD). NAFLD involves excessive accumulation of fatty acids and triglycerides in hepatocytes of non-alcohol users, driven by metabolic risk factors like obesity, type 2 diabetes, and high cholesterol. NASH advances this with hepatocyte injury, inflammatory infiltration, steatosis, fibrosis, and risks of cirrhosis or hepatocellular carcinoma.GA counters NASH by

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activating pathways that enhance lipogenesis and mitochondrial function[1]. It suppresses lipid synthesis genes, boosts β-oxidation, reduces oxidative stress via Nrf2 upregulation, and inhibits pro-inflammatory cytokines (TNF-α, IL-6) in hepatic stellate and Kupffer cells. Despite the therapeutic potential of herbal bioactives like GA for lipid regulation, their clinical use is limited by poor bioavailability and rapid metabolism. To address this, novel liposomal nanocarriers enable targeted, controlled delivery to the liver via passive and active strategies, overcoming the low efficiency of conventional systems that require high drug doses. Nanotechnology bridges this gap through liver-targeted liposomes, leveraging passive accumulation via Enhanced Permeability and Retention (EPR) effects. This study pioneers GA-loaded liposomes for precise NASH therapy, aiming to amplify efficacy, minimise off-target effects, and pave the way for herbal nanomedicine in hepatology[2-4].

2. Materials and Methods

2.1 Materials

GA, soya lecithin, cholesterol, and ethanol were purchased from Central Drug House (P) Ltd., Delhi. All other solvents and chemicals met analytical purity standards and were utilised without any additional purification.

2.2 Preparation and Characterisation of Liposome

Thin film hydration was used to create gallic acid-loaded liposomes. The required quantity of medication and the 3:1 ratio of soy lecithin to cholesterol were weighed and dissolved in ethanol. The solvent was evaporated using a rotary evaporator set at 60˚C for 15 minutes at 90 rpm after a homogeneous solution was obtained. Following evaporation, the thin film was treated with the phosphate buffer solution, centrifuged for 30 minutes at 3000 rpm, and then sonicated for 15 minutes to create liposomes[5-7].

3. Characterisation of Liposomes

3.1 Particle Size

Dynamic Light Scattering (DLS) (Litesizer, Anton Paar) was used to assess the average particle size and size distribution of liposomes loaded with gallic acid. It provides the hydrodynamic diameter of the liposomes. For this, 1ml of the sample was diluted with 3ml of phosphate-buffered saline (PBS, pH 7.4) to an optimal concentration (typically 0.01–0.1 mg/mL lipid) to minimise multiple scattering effects[8,9].

3.2 Entrapment Efficiency (%EE)

The encapsulation efficiency of liposomes containing gallic acid was assessed using the ultracentrifugation method. The liposomes were subjected to centrifugation at 6000 for 1 hour at a temperature of 4°C. This process facilitated the unloading and separation of GA liposomes. Subsequently, the liposome pellet was treated with a phosphate buffer solution. The quantity of GA was then quantified at a wavelength of 265nm. The Entrapment Efficiency (EE) was measured using the following formula: EE% = W2/W1 × 100%; where W1 represents the total weight of gallic acid added during liposome preparation, in mg; and W2 denotes the weight of Gallic acid that was encapsulated, in mg[10,11].

3.3 Experimental Design

The formulation of GA-loaded liposomes was optimised using a Box-Behnken Design (BBD) with 13 runs and three factors, each at three different levels. The independent variables, referred to as factors, included lipid concentration (X1), buffer volume (X2), and sonication duration (X3), with three levels for each variable, as shown in Table 1. The particle size (Y_1) was considered as the dependent variable[12-14].

3.4 Statistical Analysis

Stat-Ease software was used for the statistical analysis of BBD batches. The same program was used for all statistical analyses of Design of Experiments (DoE) batches. The same program was used to create response surface plots and superimposed contour plots[15-17].

3.5 Fourier Transform Infrared Spectroscopy Study

FTIR studies on GA and GA-loaded liposomes involve preparing samples by drying pure GA powder or freeze-dried liposomes into a thin film, analysing and recording spectra from 4000-400cm^{-1} at 4cm^{-1} resolution with

32-64 scans on an FTIR spectrometer (IRSpirit-X, Shimadzu), focusing on GA's characteristic peaks[18-20].

3.6 Dissolution Study

The *in vitro* dissolution testing for GA-loaded liposome and the free drug uses a common apparatus. A dissolution tester (USP) (Eletrolab) was used to assess release kinetics. Cellulose dialysis membranes 12-14 kDa were equilibrated at 4°C with water for 12 hours before the test started. The drug formula was placed on the donor side, and buffer liquid was placed on the receptor side. The experiment was run for 120 minutes at 37˚C with constant stirring at 250 rpm. At specific times, a small amount of liquid was taken from the receptor side to measure the amount of Gallic acid released using ultraviolet (UV)-Vis spectroscopy at 265nm[21-24].

4. Results and Discussion

The developed formulation was evaluated for particle size, encapsulation efficiency, and optimisation parameters from BBD, and *in vitro* drug release kinetics

4.1 Physical Characterisation of Gallic Acid-loaded Liposome

4.1.1 Particle Size

Particle size of GA-liposome was determined through DLS (Litesizer, Anton Paar) with an average particle size 2.7µm as shown in Figure 1. The particle size in the micrometre range, with a uniform size distribution, indicates the successful formulation of a liposome.

4.1.2 Drug Entrapment Efficiency

The encapsulation efficiency of 80% suggests effective drug entrapment within the liposome, which may improve drug stability and controlled drug release

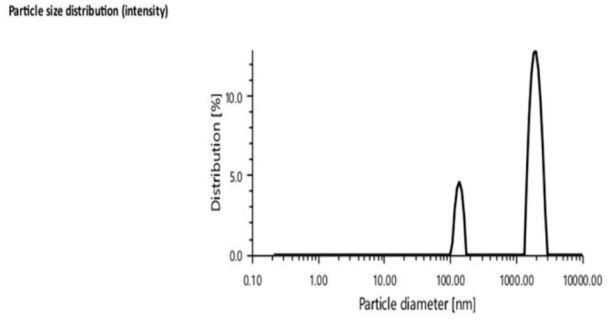


Figure 1. Particle size distribution.

properties. It will enhance GA stability against hepatic metabolism and rapid clearance, sustained release profiles, and improved pharmacokinetics for liver targeting.

4.1.3 Optimisation

The GA-loaded liposome formulation was optimised using a 13-run, 3-factor, 3-level BBD. Depending on the levels selected for the independent variables, there were thirteen formulations Table 1. After that, the thirteen formulations were created and evaluated based on the chosen particle size responses. Table 2 provides a full analysis of the outcomes. The particle size responses ranged from 1µm to 4µm, suggesting that the factor values selected were suitable to produce the desired results. Table 3 shows the statistical analysis of the chosen criteria. The model is considered significant based on its F value of 5.42. An F value this large has a 2.09% probability of being caused by noise. Significant model terms are indicated by p-values less than 0.05. Case A is the important mode in this instance. The response plots, which include contour plots and 3D surface plots Particle size results were shown to be directly correlated with lipid concentration, as depicted in Figures 2 and 3.

With sonication time (factor C) fixed at its central level 0, 3D response surface plots were generated via quadratic regression from the ANOVA data. 3D visualisations map how lipid concentration (A) and buffer volume (B) jointly influence the particle size (Figure 1).

The final optimisation equation obtained from ANOVA for particle size in terms of coded factors was: $Y=b_0 + 0.753A + 0.223C$, indicating that lipid concentration and sonication time significantly influenced particle size

The value of the y-intercept is $b_0=0.024$

4.1.4 FTIR Study

FTIR analysis of GA and GA-loaded liposomes reveals characteristic spectral signatures confirming successful encapsulation without chemical degradation. Pure GA exhibits distinct peaks including a broad O-H stretching band at 3400-3200cm^{-1} from phenolic hydroxyls, aromatic C-H stretch at 3100-3000cm^{-1}, C=O carbonyl stretch at 1700-1720cm^{-1}, aromatic C=C vibrations at 1600-1450cm^{-1}, and C-O phenolic stretches at 1200-1100cm^{-1} as shown in Figure 4a, and in the liposomal

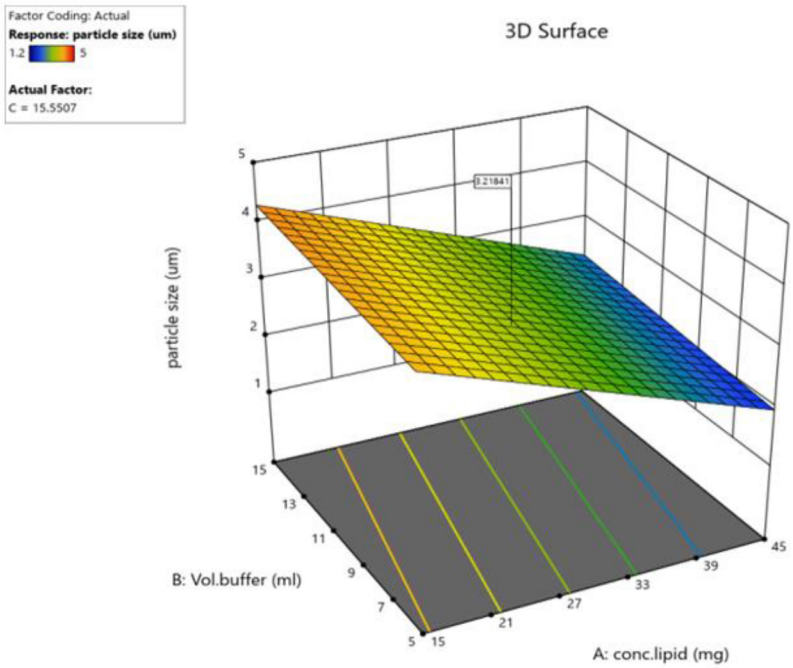


Figure 2. The response surface plots illustrate how different factors interact to affect a specific outcome in three dimensions for each dependent variable according to the response degree (light green- lowest response level and dark red -highest response level). (X_1): lipid concentration; (X_2): volume of buffer; and (X_3): sonication time; (Y_1) is the response particle size.

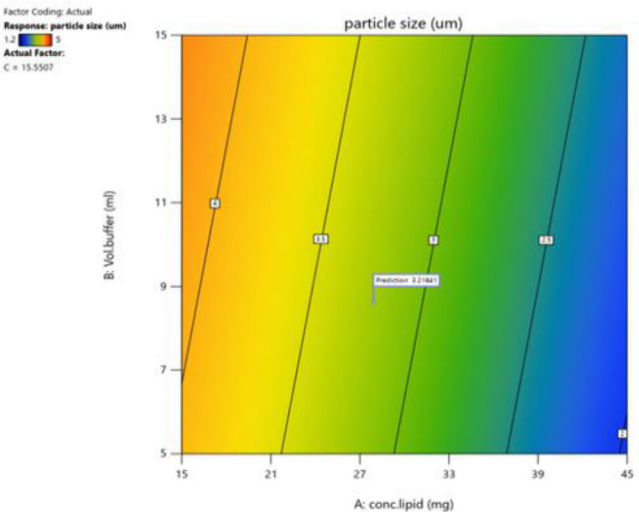


Figure 3. A contour plot illustrating how the independent variables affect particle size.

spectrum shown in Figure 4b due to hydrogen bonding between GA's polar groups and phospholipid head groups like soyalecithin Liposome-specific bands appear at CH_2 asymmetric/symmetric stretches (2920/2850cm^{-1}), C=O ester (1740cm^{-1}), and PO_2^- asymmetric (1220cm^{-1}), showing minor intensity increases or red-shifts in the

Table 1. Formulation variables and their levels

Independent Variable		Levels	
	-1	0	1
Conc. of lipid (mg)	15	30	45
Volume of buffer (mL)	5	10	15
Sonication time (min.)	15	22.5	30

GA-loaded formulation, indicating physical entrapment within the bilayer's polar interface rather than disruption, with no new peaks signifying stable molecular integrity. These overlaid spectra demonstrate GA's integration into the lipid matrix, supporting enhanced formulation stability for liver-targeted delivery in NASH applications.

4.1.5 Comparison of Drug and Formulation Dissolution

To assess the *in vitro* behaviour of the optimised batch acquired following DoE experiments, *in vitro* drug release testing was carried out.

The result shows that drug dissolution with respect to time in Gallic acid solution is less than the drug permeability in GA-loaded liposome, as reflected in

Table 2. The formulations created for the Box-Behnken factorial design for Gallic acid are described in detail, along with the corresponding physical characterisation

Samples	(X_1) Lipid Concentration (mg)	(X_2) Volume of buffer (mL)	(X_3) Sonication Time (min)	(Y_1) Particle Size (µm)
1	15	5	22.5	2.3
2	30	5	15	3.3
3	15	10	30	3.7
4	45	5	22.5	2.7
5	45	15	22.5	2
6	45	10	30	1.2
7	45	10	15	1.4
8	30	15	30	2
9	30	10	22.5	1.9
10	30	5	30	1.5
11	30	15	15	3
12	15	15	22.5	4.2
13	15	10	15	5

Table 3. ANOVA analysis of response: Particle size

Source	Sum of squares	df	Mean Square	F-value	P-value	
Model	10.36	3	3.45	5.42	0.0209	significant
A-conc. of lipid	7.80	1	7.80	12.25	0.0067	
B-vol. of buffer	0.2450	1	0.2450	0.3848	0.5504	
c-sonication time	2.31	1	2.31	3.63	0.0891	
Residual	5.73	9	0.6367			
Cor total	16.09	12				

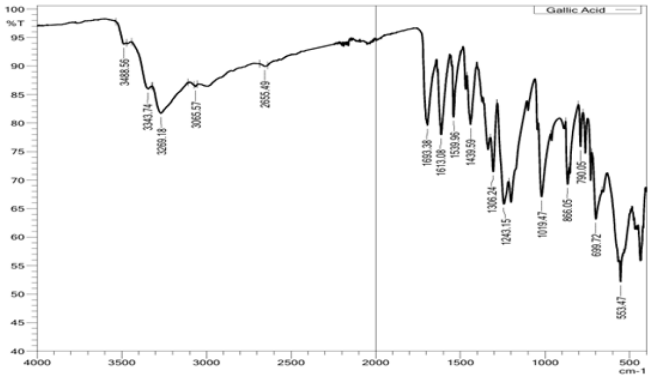


Figure 4a. FTIR spectrum of gallic acid: C-H stretch at 3100-3000cm^{-1}, C=O carbonyl stretch at 1700-1720cm^{-1}, aromatic C=C vibrations at 1600-1450cm^{-1}, and C-O phenolic stretches at 1200-1100cm^{-1}.

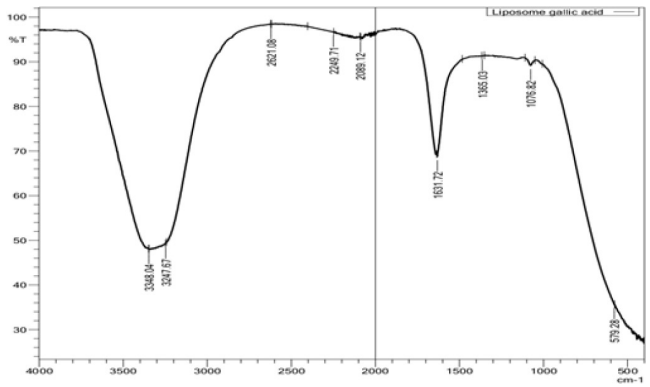


Figure 4b. FTIR spectrum of Gallic acid-loaded liposome.

Figure 5. In the GA solution, drug dissolution across the membrane increases rapidly with time due to its free diffusion, driven by high aqueous solubility but limited by poor lipophilicity and rapid clearance, resulting in suboptimal sustained release. Conversely, GA-loaded liposomes exhibit lower overall dissolution

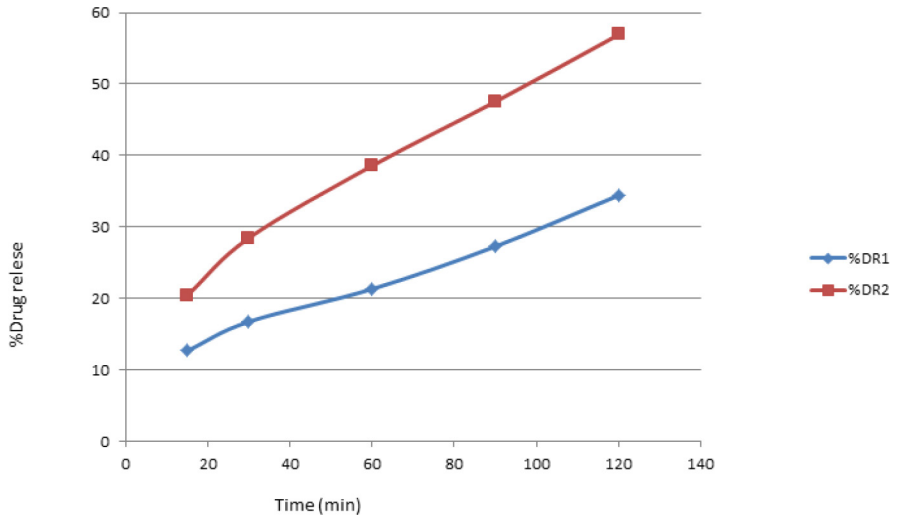


Figure 5. % Drug release of Gallic acid % (DR1) and Gallic acid loaded liposome % (DR2).

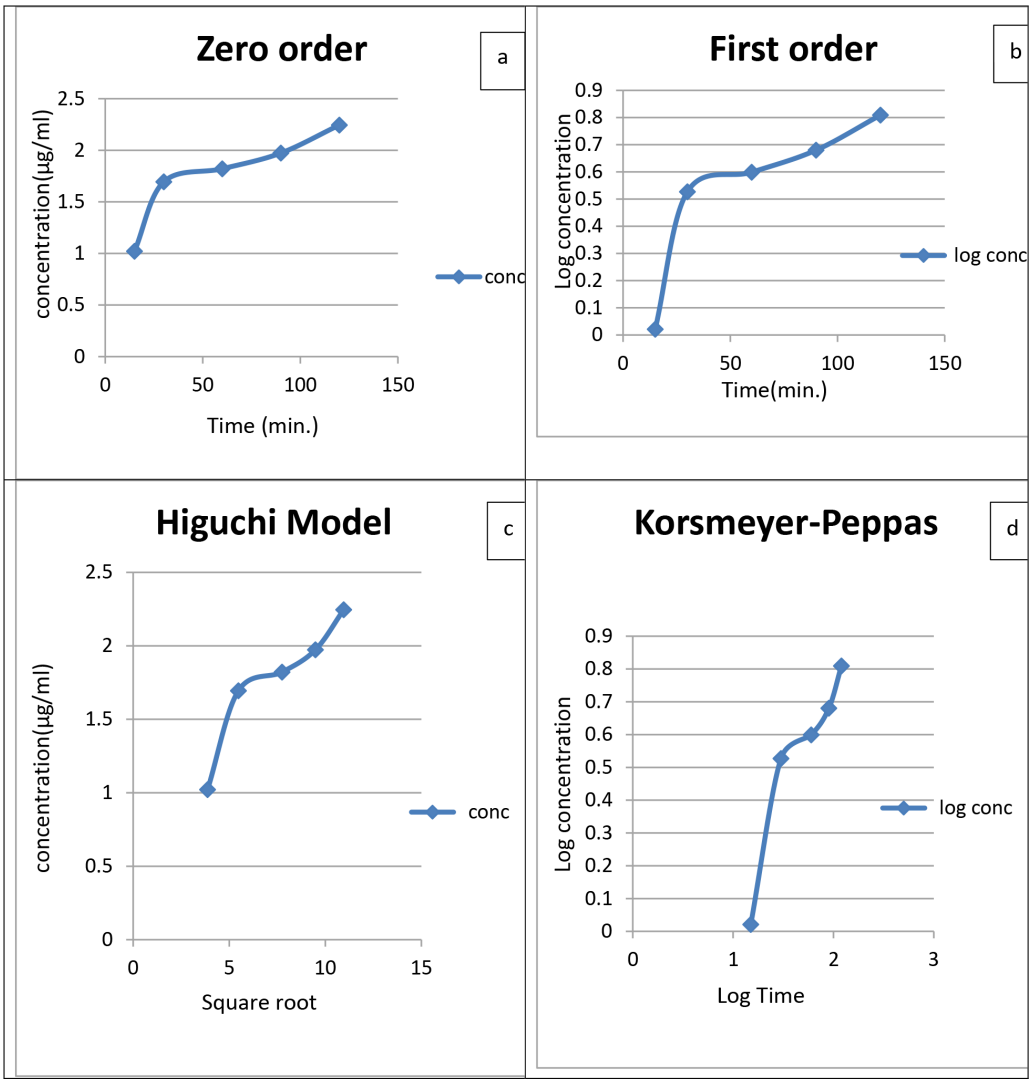


Figure 6. **(a).** Zero-order graph, **(b).** First-order graph, **(c).** Higuchi graph, **(d).** Korsmeyer-Peppas graph.

over the same timeframe, as the lipid bilayer structure controls release through a biphasic mechanism: an initial burst from surface-bound drug, followed by slower diffusion from the inner core, enhancing stability, prolonging circulation, and improving targeted delivery to sites like the liver while minimising premature systemic exposure. This controlled profile reduces peak concentrations that could cause toxicity, making liposomes superior for therapeutic applications in conditions like oxidative stress-related liver diseases.

4.1.6 Kinetics of Drug Release

The drug release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. The R^2 values obtained were 0.958, 0.914, 0.982, and 0.967, respectively. Since the Higuchi model showed the highest R^2 value (0.982), the drug release follows the Higuchi diffusion-controlled release mechanism, as depicted in Figure 6 (a-d).

5. Conclusion

This paper describes the effective optimisation of a liposome formulation loaded with gallic acid. Additionally, it was demonstrated that BBD is an effective experimental design to optimise vesicular systems for the administration of gallic acid. The optimised formulation exhibited an average particle size of approximately 2.7µm with a drug encapsulation efficiency of 80%; FTIR study of pure gallic acid and the formulation showed its identity and stability. *In vitro* dissolution studies using the Higuchi model showed the highest R^2 value (0.982); the drug release follows Higuchi diffusion controlled release mechanism further revealed that the gallic acid-loaded liposomes exhibited enhanced drug dissolution compared to the free drug solution, suggesting improved release characteristics and bioavailability. Overall, the findings highlight that liposomal encapsulation of gallic acid is a promising strategy for targeted delivery in the management of non-alcoholic Steatohepatitis, with improved stability, dissolution, and therapeutic potential.

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Author Contribution

RPM conceived of the present idea for the article. PM performed the literature search, data analysis, and wrote the manuscript. SP and RKM provided critical feedback and helped to shape the final draft. All authors have read and agreed to the published version of the manuscript.