RESEARCH PAPER

Phytosomal Encapsulation of Epigallocatechin Gallate: A Strategy for Enhanced Bioavailability and Neuroprotective Potential in Parkinson's disease Management

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Abstract

Background: Epigallocatechin gallate (EGCG), a major catechin derived from *Camellia sinensis*, is extensively studied for its neuroprotective properties, including antioxidative and anti-inflammatory effects, making it a promising candidate for Parkinson's disease (PD) management. However, its therapeutic application is severely limited by low systemic bioavailability and poor chemical stability.

Objective: This study aimed to develop and characterize EGCG phytosomes as a novel delivery system to overcome the inherent biopharmaceutical limitations of EGCG, thereby enhancing its therapeutic potential for PD.

Methods: EGCG was extracted from green tea using Soxhlet extraction with an ethanol:ethyl acetate (70:30 v/v) solvent system. Phytosomes were subsequently prepared using soya lecithin and cholesterol, and the formulation was systematically optimized using a full factorial Design of Experiments (DoE), evaluating EGCG:lecithin ratio and cholesterol concentration. The optimized batch was thoroughly characterized for particle size, zeta potential, entrapment efficiency (EE), and thermal behavior using Differential Scanning Calorimetry (DSC).

Results: The optimized EGCG phytosomes exhibited uniform, nanoscale vesicular size (90.5 nm), a high entrapment efficiency of 87.0%, and a stable zeta potential of -29.12 mV. DSC analysis confirmed the successful formation of an EGCG-phospholipid complex, indicated by a significant shift in the melting endotherm of EGCG. Morphological analysis via Transmission Electron Microscopy (TEM) revealed well-defined, spherical vesicles. Stability studies showed promising retention of EE over one month.

Conclusion: Phytosomal encapsulation successfully enhances EGCG stability and drug loading, offering a superior delivery system compared to free EGCG. These EGCG phytosomes represent a promising, natural, and multi-faceted approach for targeted neuroprotection in Parkinson's disease, warranting further *in vivo* validation for their neuroprotective efficacy and behavioral outcomes.

Keywords: EGCG, Phytosomes, Parkinson's disease, Soya lecithin, Cholesterol, Bioavailability Enhancement.

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1. Introduction

Parkinson's disease (PD) is a long-term, progressive neurodegenerative condition that presents significant global social, psychological, and economic challenges [1]. Although traditionally defined by its motor symptoms (MSs) such as tremors, rigidity, bradykinesia, and postural instability, PD encompasses a broader spectrum of non-motor symptoms (NMSs) that profoundly diminish patients' overall quality of life [2]. Worldwide, PD ranks as the second most common neurodegenerative disorder after Alzheimer's disease, affecting more than 10 million individuals, predominantly in middle-aged and elderly populations [3, 4]. As the disorder progresses, non-motor manifestations—including cognitive decline, mood disturbances, sleep irregularities, and autonomic dysfunction—tend to emerge, compounding the physical and emotional burden of the disease and often having a more profound adverse effect on daily functioning and

economic outcomes than motor symptoms themselves [5, 6].

Neuropathologically, PD is distinguished by the progressive degeneration of dopaminergic neurons within the substantia nigra pars compacta, leading to dopamine depletion in the striatum [7]. This neurotransmitter imbalance interferes with basal ganglia circuitry and gives rise to characteristic motor impairments. Furthermore, the involvement of other neuronal systems—including serotonergic, noradrenergic, and cholinergic networks—accounts for the diverse range of non-motor disturbances observed in PD [8]. At the cellular and molecular levels, PD pathogenesis is complex and multifactorial, involving mitochondrial dysfunction, oxidative and nitrosative stress, abnormal protein folding, and impairment of protein degradation systems [9, 10]. The aggregation of misfolded -synuclein into Lewy bodies represents a pathological hallmark of PD, while mitochondrial failure and oxidative damage accelerate neuronal degeneration [11,12]. Collectively, these interrelated mechanisms contribute to the gradual and disabling course of the disease [13].

Epigallocatechin-3-gallate (EGCG) is the principal and most bioactive catechin present in green tea (*Camellia sinensis*), comprising approximately 50–60% of its total polyphenolic constituents [14]. Recognized for its potent antioxidant and neuroprotective properties, EGCG has garnered significant scientific interest for its potential role in combating neurodegenerative diseases, particularly PD [15,16]. Owing to its unique polyphenolic structure, EGCG effectively scavenges reactive oxygen species (ROS), chelates transition metal ions, and modulates various intracellular signaling pathways essential for neuronal protection and survival [17]. EGCG has been shown to mitigate pathogenic mechanisms by preventing -synuclein fibril formation, enhancing mitochondrial efficiency, and boosting endogenous antioxidant systems such as superoxide dismutase and glutathione peroxidase [18,19].

Beyond its antioxidant potential, EGCG also demonstrates anti-inflammatory capabilities by inhibiting microglial activation and reducing the release of pro-inflammatory cytokines—processes that otherwise contribute to dopaminergic neuronal damage in the substantia nigra [20,21]. Experimental research employing PD models, such as 6-hydroxydopamine (6-OHDA) and MPTP-induced neurotoxicity, further supports EGCG's neuroprotective efficacy through improvements in motor coordination, preservation of dopaminergic neurons, and restoration of neurochemical homeostasis within the basal ganglia [22,23]. Given its diverse pharmacological profile—combining antioxidant, anti-apoptotic, and anti-inflammatory mechanisms—EGCG stands out as a promising phytoconstituent for the prevention and management of PD [24].

Parkinson's disease is primarily managed with conventional medications such as levodopa, dopamine agonists, monoamine oxidase-B (MAO-B) inhibitors, and catechol-O-methyltransferase (COMT) inhibitors. Although these pharmacological agents provide symptomatic improvement, their prolonged administration often results in significant adverse reactions, including dyskinesia, motor fluctuations, gastrointestinal discomfort, and neuropsychiatric disturbances [25,26]. Furthermore, these therapies fail to prevent or reverse neuronal degeneration, as they act mainly by compensating for dopaminergic loss rather than addressing the underlying pathophysiological mechanisms [27]. This limitation has encouraged exploration of natural compounds with neuroprotective potential, such as EGCG [28].

EGCG provides several therapeutic benefits compared to conventional allopathic drugs owing to its potent antioxidant, anti-inflammatory, and anti-apoptotic activities, which directly target the core molecular pathways involved in PD progression [29]. Unlike synthetic agents that primarily modulate dopaminergic signaling, EGCG can cross the blood–brain barrier (BBB) and protect dopaminergic neurons from oxidative stress, mitochondrial dysfunction, and -synuclein aggregation [30,31]. It also enhances the brain's intrinsic defense mechanisms, including glutathione and superoxide dismutase activity [32, 33]. Moreover, EGCG exhibits an excellent safety margin compared to long-term dopaminergic therapy, with experimental evidence suggesting that it confers neuroprotection without causing tolerance, dependency, or notable systemic toxicity [34, 35]. However, the therapeutic potential of EGCG is significantly hampered by its poor oral bioavailability, rapid metabolism, and low stability under physiological conditions [36, 37]. This necessitates the development of advanced drug delivery systems to improve its pharmacokinetic profile and enhance its efficacy in neurological disorders [38].

Phytosomes, which are lipid-compatible molecular complexes of phytoconstituents with phospholipids, represent a promising approach to overcome these limitations. They improve the absorption and cellular uptake of herbal extracts by facilitating their passage

across biological membranes, thereby enhancing bioavailability and therapeutic efficacy [39, 40]. The aim of this study was, therefore, to develop and characterize EGCG phytosomes using a systematic Design of Experiments (DoE) approach, to enhance its bioavailability and stability for potential application in Parkinson's disease management. This research sought to identify optimal formulation parameters that yield EGCG phytosomes with desirable physicochemical characteristics, ultimately paving the way for a more effective and safe therapeutic strategy against PD [41, 42].

2. Material and Methods

2.1. Materials

Tetley green tea powder (Tetley, India) was utilized for the extraction of Epigallocatechin. Standard Epigallocatechin gallate was procured from Yucca Enterprises, Mumbai. Phospholipid: Phosphatidylcholine (PC) soya lecithin and Cholesterol were obtained from Modern Industries C-17 MIDC, Malegaon-Sinnar, Dist. Nashik. All other chemicals and reagents used were of analytical grade.

2.2. Methods

2.2.1. Extraction of Epigallocatechin Gallate from Green Tea

A total of 100 g of green tea powder was placed in a Soxhlet extractor and extracted with 200 mL of a solvent mixture of ethanol and ethyl acetate (70:30 v/v) [43]. The extraction was performed at a controlled temperature of 60–70 °C for multiple cycles until the plant material was completely exhausted, as indicated by a clear siphoning solvent. The combined extract was concentrated under reduced pressure using a rotary evaporator to remove the solvents, yielding a green tea extract. This extract was then stored in an airtight container at 4 °C until further use in phytosome preparation.

2.2.2. Procedure for the Preparation of EGCG Phytosomes

EGCG phytosomes were prepared using a modified solvent evaporation method [44]. Accurately weighed amounts of EGCG, lecithin, and cholesterol were taken. EGCG was dissolved in ethanol, while lecithin and cholesterol were dissolved in chloroform. Both solutions were then mixed thoroughly, and the resulting mixture was sonicated for 10 minutes to ensure complete miscibility and initial complex formation. The solvent was subsequently removed using a rotary evaporator at 30–40 °C under reduced pressure, leading to the formation of a thin lipid-drug layer on the flask wall. This thin layer was then hydrated with phosphate buffer solution (pH 7.4), followed by a final sonication step to yield a homogenous phytosome dispersion. The prepared phytosomes were stored at 2°C –4 °C until further use to maintain their stability.

2.2.3. Epigallocatechin Gallate Phytosomes Batches Design by DoE

Design of Experiment (DoE) was employed to systematically evaluate the impact of formulation variables on the quality attributes of the EGCG phytosomal preparation [45]. A full factorial experimental design () was selected to study the influence of two independent factors: EGCG:Lecithin ratio (at three levels: 1:1, 1:2, and 1:3) and Cholesterol concentration (at three levels: 1%, 5%, and 9%) on the dependent variables, namely average particle size and entrapment efficiency of the phytosome batches. Table 1 outlines the design matrix for the nine experimental batches.

Table 1: Phytosomes Batches Design (DOE)

Batch No.	EGCG: Lecithin ratio	Drug Added (mg)	Lecithin (mg)	Cholesterol (mg)	Total Weight (mg)
F1	1:3	50	150	1.5	201.5
F2	1:1	50	50	7.5	107.5
F3	1:2	50	100	7.5	157.5
F4	1:3	50	150	7.5	207.5
F5	1:3	50	150	13.5	213.5
F6	1:1	50	50	13.5	113.5
F7	1:1	50	50	1.5	101.5
F8	1:2	50	100	13.5	163.5
F9	1:2	50	100	1.5	151.5

2.2.4. Characterization Parameters of Epigallocatechin-3-gallate Phytosomes Batches

1. Zeta Potential Measurement

The zeta potential of the EGCG phytosome batches was measured to assess their surface charge and colloidal stability [46]. Analysis was performed using a zeta potential analyzer based on the Dynamic Light Scattering (DLS) technique (Malvern Zetasizer Nano ZS) at a controlled temperature of °C. Before measurement, the phytosome dispersions were suitably diluted with double-distilled water to obtain optimal scattering conditions and prevent multiple scattering effects. Zeta potential values exceeding mV were interpreted as indicative of good electrostatic repulsion between vesicles, signifying enhanced stability and minimal aggregation, which is crucial for prolonged circulation and reduced clearance *in vivo* [47].

2. Entrapment Efficiency (EE) Determination

The entrapment efficiency of EGCG phytosome batches was evaluated using a UV–visible spectrophotometric method [48]. An accurately measured volume of the phytosome dispersion was centrifuged at 10,000 rpm for 30 minutes at 4°C to separate the unentrapped EGCG from the vesicular fraction. The supernatant, containing the free (unentrapped) drug, was collected, appropriately diluted with phosphate buffer (pH 7.4), and analyzed at a wavelength of 275 nm using a UV–visible spectrophotometer (Shimadzu UV-1800). The concentration of unentrapped EGCG was calculated from a previously established standard calibration curve of EGCG in phosphate buffer. The percentage entrapment efficiency was then calculated using the following formula:

2.2.5. Evaluation of Optimized Batch

Morphological Analysis by Transmission Electron Microscopy (TEM)

The morphology and structural features of the optimized EGCG phytosome formulation were analyzed using Transmission Electron Microscopy (TEM) [49]. A small amount of the phytosome suspension was diluted with distilled water and carefully placed onto a carbon-coated copper grid. The sample was allowed to stand for a few minutes to facilitate vesicle adsorption, and excess liquid was gently removed with filter paper. The grid was then negatively stained with 1% phosphotungstic acid to provide image contrast. After complete air drying, the sample was examined under a TEM (Thermofisher Talos L120C G2) at an accelerating voltage of 120 KV at suitable magnifications to observe the vesicle size, shape, and dispersion uniformity.

Differential Scanning Calorimetry (DSC) Analysis

The optimized EGCG phytosome batch was subjected to Differential Scanning Calorimetry (DSC) to evaluate its thermal characteristics and possible interactions between EGCG and formulation components [50]. Precisely weighed samples (2-5 mg) of pure EGCG, the physical mixture of excipients, and the optimized phytosome formulation were hermetically sealed in aluminum pans. The analysis was performed using a DSC instrument (PERKIN ELMER, Model: PYRIS 6 DSC) under a nitrogen purge at a flow rate of 20 mL/min to maintain an inert atmosphere. Samples were heated from 30 °C to 300 °C at a constant heating rate of 10 °C/min, and thermograms were recorded. Shifts, broadening, or disappearance of endothermic and exothermic peaks were analyzed to confirm drug–excipient compatibility and successful phytosome formation, particularly the complexation between EGCG and phospholipids [51].

Zeta Potential of Optimized EGCG Phytosomes Batch

The surface charge and stability of the optimized EGCG phytosome batch were reassessed by measuring its zeta potential using the DLS method at °C. The phytosome dispersion was appropriately diluted with double-distilled water. Measurements were performed in triplicate, and the mean value was reported, alongside the Polydispersity Index (PDI) to assess particle size homogeneity [47].

Stability Testing

A stability study of the phytosomal optimized batch of EGCG was performed under accelerated storage conditions. Samples were stored at °C and relative humidity (RH) for a period of one month [52]. Entrapment efficiency data at the initial stage and after 1 month were determined to monitor the physical stability and drug retention capabilities of the formulation.

Statistical Analysis

Statistical analysis for the Design of Experiments was performed using Design Expert software (version 12, Stat-Ease, Inc., Minneapolis, MN, USA). This software was used to generate response surface plots, analyze variance (ANOVA), and validate the experimental model by comparing predicted values with actual experimental results.

3. Results

Zeta Potential and Particle Size of Phytosome Batches

The physicochemical properties of the nine EGCG phytosome batches, including particle size and zeta potential, were systematically evaluated (Table 2). The particle sizes ranged from 90.5 nm to 160.45 nm, demonstrating that all formulations were successfully prepared within the nanoscale range, which is critical for enhanced cellular uptake and improved bioavailability [41]. Zeta potential values varied from -20.13 mV to -29.12 mV, indicating a consistent negative surface charge across the batches. Figure 1 graphically presents the zeta potential studies, showing the relatively uniform and stable surface charge distribution among the different formulations.

Table 2: Zeta Potential and Particle Size of Phytosomes Batches

Batch	EGCG: Lecithin	Drug Ad	Lecithin (mg)	Cholesterol (mg)	Total Wt	Particle	Zeta potential

No.	thin ratio	ded (mg)	)		ight (mg)	size (nm)	(mV)
F1	1:3	50	150	1.5	201.5	104.89	-28.2
F2	1:1	50	50	7.5	107.5	152.9	-21.6
F3	1:2	50	100	7.5	157.5	125.9	-26.6
F4	1:3	50	150	7.5	207.5	100.3	-28.92
F5	1:3	50	150	13.5	213.5	90.5	-29.12
F6	1:1	50	50	13.5	113.5	130.6	-20.13
F7	1:1	50	50	1.5	101.5	160.45	-20.72
F8	1:2	50	100	13.5	163.5	117.3	-25.36
F9	1:2	50	100	1.5	151.5	130.4	-27.89

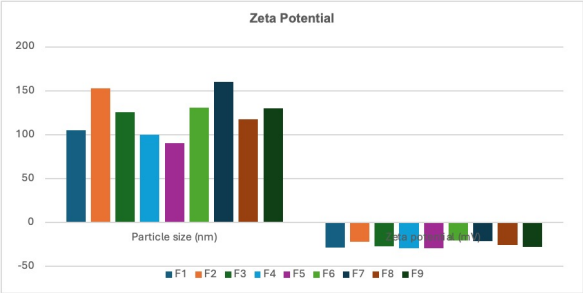

Figure 1: Zeta Potential Studies of all 9 batches of EGCG Phytosomes

The EGCG phytosome batches were evaluated for particle size and zeta potential to assess their physical characteristics and stability. Particle size measurements indicated that the phytosomes were within the nanoscale range with a fairly uniform distribution, reflecting a homogeneous formulation. Differences in particle size and zeta potential among batches were attributed to the EGCG-to-lecithin ratio and cholesterol content. As observed, batches with higher lecithin ratios (e.g., F1, F4, F5) and certain cholesterol concentrations tended to yield smaller particle sizes and more negative zeta potentials, suggesting improved colloidal stability.

Entrapment Efficiency of Phytosome Batches

The entrapment efficiency (EE) of the prepared EGCG phytosome batches was determined to quantify the extent of EGCG incorporation within the lipid vesicles (Table 3). The results showed a significant proportion of EGCG was successfully encapsulated, with EE ranging from 72.9% (Batch F2) to 87.0% (Batch F5). Figure 2 illustrates the cumulative percentage entrapment efficiency, highlighting the variations among different batches based on the EGCG-to-lecithin ratio and cholesterol content. Specifically, formulations with a higher lecithin content (1:3 ratio, e.g., F1, F4, F5) generally improved drug entrapment by providing a more extensive lipid matrix for EGCG complexation. This indicates that the lipid components effectively formed a matrix capable of encapsulating the EGCG molecules. Overall, the observed entrapment efficiency indicates effective loading of EGCG into the phytosomes, which is expected to enhance its stability and bioavailability by protecting it from degradation and improving its passage across biological barriers.

Table 3: Entrapment Efficiency of Phytosomes Batches

Batch No.	EGCG: Lecithin ratio	Drug Added (mg)	Lecithin (mg)	Cholesterol (mg)	Sample Absorbance (A)	Diluted Conc. (µg/mL)	Corrected Conc. (µg/mL, ×10)	Free Drug (mg in 10 mL)	Entrapped Drug (mg)	EE (%)
F1	1:3	50	150	1.5	1.421	81.88	818.8	8.19	41.81	83.6
F2	1:1	50	50	7.5	2.1	135.70	1357.0	13.57	36.43	72.9
F3	1:2	50	100	7.5	1.740	107.14	1071.4	10.71	39.29	78.6
F4	1:3	50	150	7.5	1.32	73.81	738.1	7.38	42.62	85.2
F5	1:3	50	150	13.5	1.201	64.90	649.0	6.49	43.51	87.0
F6	1:1	50	50	13.5	1.89	119.00	1190.0	11.90	38.10	76.2
F7	1:1	50	50	1.5	1.92	121.39	1213.9	12.14	37.86	75.7
F8	1:2	50	100	13.5	1.47	86.98	869.8	8.70	41.30	82.6
F9	1:2	50	100	1.5	1.78	113.21	1132.1	11.32	38.68	77.4

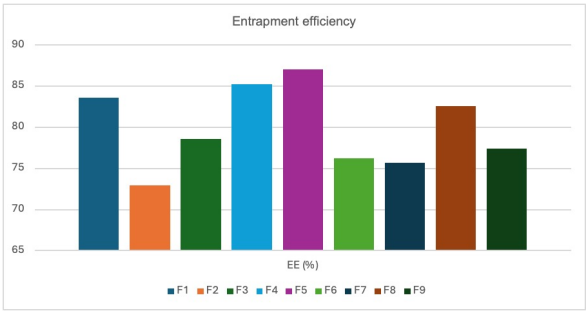

Figure 2: Cumulative Percentage Entrapment Efficiency Studies of all 9 batches of EGCG Phytosomes

Optimized Batch and Design of Experiment (DoE) Validation

Among the various formulations prepared, Batch F5 was identified as the optimized batch based on its superior physicochemical profile. This batch, formulated with an EGCG-to-lecithin ratio of 1:3 and containing 13.5 mg of cholesterol, demonstrated the smallest particle size (90.5 nm), the highest entrapment efficiency (87.0%), and a highly stable zeta potential (-29.12 mV). The higher lecithin concentration in Batch F5 evidently provided a more extensive lipid matrix, facilitating better encapsulation of EGCG. Concurrently, the optimal cholesterol content contributed to enhanced vesicle rigidity and overall formulation stability. These characteristics highlight Batch F5 as exhibiting superior formulation attributes, making it the most suitable candidate for further in-depth evaluation and development.

The reliability of the Design of Experiment (DoE) model used for optimization was confirmed by plotting the experimental values against the model predictions. As shown in Figure 3(A) and 3(B), the data points are closely aligned with the regression line, indicating a strong agreement between the observed experimental results and the values predicted by the model. This close correlation validates the predictive capability of the Design Expert model, confirming its accuracy in describing the relationship between the formulation factors (EGCG:Lecithin ratio and Cholesterol concentration) and the measured responses (particle size and entrapment efficiency). The response surface analysis, implicitly represented by this agreement, suggests that the model effectively captured the complex interactions between these variables, leading to a robust optimization process for the EGCG phytosomes.

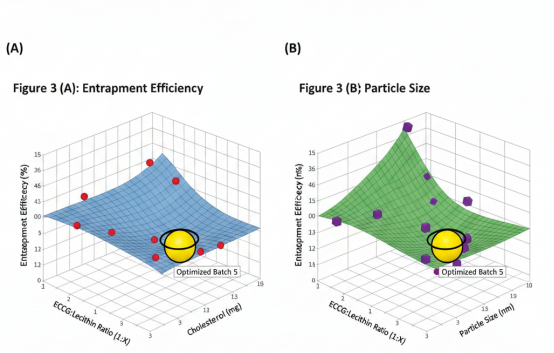

Figure 3 (A & B): The plot (A) and (B) indicates that the experimental values are in close agreement with the model predictions, confirming the reliability of the Design Expert model.

Morphological Analysis of Optimized EGCG Phytosomes by Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) was performed to visualize the morphology and confirm the nanoscale dimensions of the optimized EGCG phytosomes (Batch F5). The obtained TEM images (Figure 4) reveal well-defined, spherical to nearly spherical vesicles with smooth surfaces. The phytosomes appeared uniformly distributed, with average particle sizes ranging approximately from 70-100 nm. This observation aligns well with the DLS measurements and confirms the successful formation of nanometer-sized colloidal particles, indicating good homogeneity and stability of the formulation. The distinct morphology and uniform size distribution are crucial for predictable *in vivo* performance and enhanced cellular interaction.

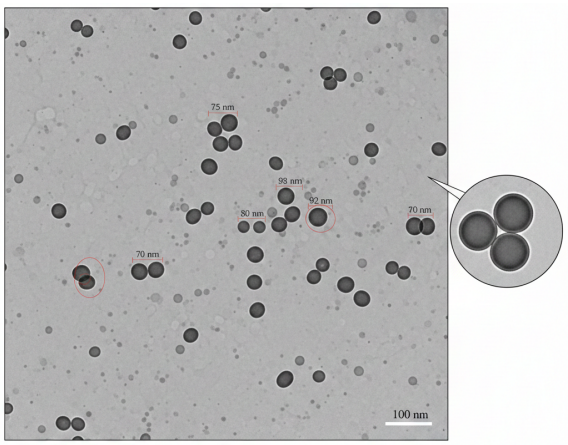

Figure 4: The obtained TEM images reveal well-defined, spherical to nearly spherical vesicles with smooth surfaces. The phytosomes appeared uniformly distributed with average particle size 70-100 nm, confirming the nanoscale morphology and stability of the formulation.

Differential Scanning Calorimetry (DSC) Analysis of Optimized EGCG Phytosomes Batch

Differential Scanning Calorimetry (DSC) was conducted to investigate the thermal behavior of pure EGCG and the optimized phytosome batch, thereby providing insights into the drug-excipient interactions and complexation [50]. The DSC thermogram of pure EGCG (Figure 5) exhibited a sharp endothermic peak at approximately 225 °C, corresponding to its crystalline melting point. In contrast, the DSC thermogram of the optimized EGCG phytosome batch (Figure 6) showed a significant shift and attenuation of this characteristic EGCG peak, which appeared as a broader, less intense endotherm around 118 °C. This distinct alteration in the thermal profile of EGCG within the phytosome complex, compared to its pure form, indicates a strong molecular interaction between EGCG and the phospholipids, leading to the successful formation of an EGCG-phospholipid complex (phytosome) rather than a simple physical mixture. The disappearance of the sharp EGCG peak suggests a loss of its crystalline structure and its incorporation into the amorphous lipid matrix of the phytosome.

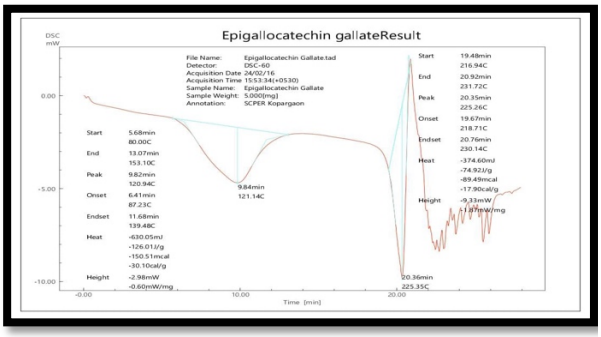

Figure 5: Pure EGCG: Sharp endothermic peak around 225°C

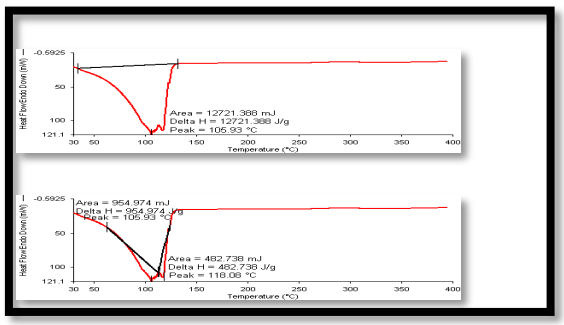

Figure 6: The DSC peak of optimized phytosomes batch shift at 118 °C means optimized EGCG-phospholipid complex (phytosome) is successfully formed

Zeta Potential of Optimized EGCG Phytosomes Batch

The zeta potential of the optimized phytosome formulation (Batch F5) was measured to further confirm its colloidal stability (Figure 7). The optimized formulation exhibited a zeta potential value of -29.12 mV. This value, being close to mV, indicates good electrostatic repulsion among the particles, which is critical for maintaining colloidal stability and preventing aggregation in suspension. Furthermore, the Polydispersity Index (PDI) was determined to be 0.1611, signifying a narrow particle size distribution. A low PDI value confirms that the phytosomes are highly uniform and homogeneous in size, contributing to predictable *in vivo* performance and consistent drug release.

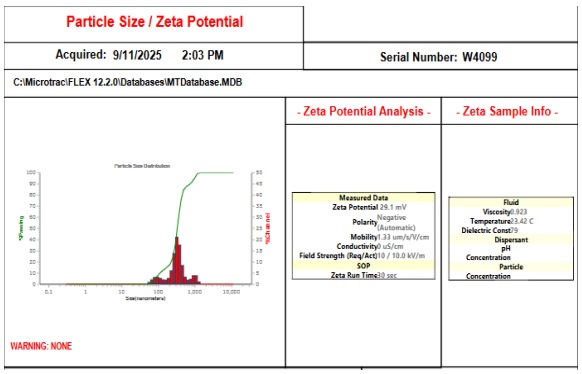

Figure 7: Zeta potential distribution graph of the optimized EGCG phytosome batch

Stability Study

The physical stability of the formulated optimized batch of EGCG Phytosomes (Batch F5) was monitored for one month under ambient storage conditions (°C and RH). Batch F5 initially displayed the highest entrapment efficiency (87.0%). After one month of storage, the entrapment efficiency slightly decreased to 85.0%. This minimal reduction (only 2%) in EE over the storage period indicates excellent physical stability and retention of the encapsulated EGCG within the phytosomal matrix. The results suggest that the formulation components, particularly the cholesterol, contributed effectively to membrane rigidity, thereby minimizing drug leaching and maintaining the integrity of the phytosomes during storage.

4. Discussion

The development of effective drug delivery systems for neuroprotective agents is paramount in the management of complex neurological disorders like Parkinson's disease [11, 41]. This study successfully demonstrated the formulation and optimization of EGCG phytosomes, addressing the inherent limitations of EGCG related to its poor bioavailability and chemical instability [36, 37]. The rationale for employing a phytosomal strategy lies in its ability to

enhance the lipophilicity of polar compounds like EGCG, thereby facilitating their transport across biological membranes, including the blood-brain barrier (BBB), and improving cellular uptake [39, 40].

The systematic optimization using a full factorial Design of Experiments proved instrumental in identifying the most influential formulation parameters. As evidenced by the results, the EGCG:Lecithin ratio and cholesterol concentration significantly impacted critical quality attributes, namely particle size and entrapment efficiency. Batch F5, characterized by an EGCG:Lecithin ratio of 1:3 and 13.5 mg cholesterol, emerged as the optimized formulation due to its superior physicochemical profile. The higher lecithin content in F5 likely provided a greater number of phospholipid molecules for complexation with EGCG, leading to higher entrapment efficiency (87.0%) [42]. Lecithin's amphiphilic nature enables it to form stable complexes with EGCG, enhancing its encapsulation and protecting it from enzymatic degradation and oxidation [29].

The nanoscale particle size (90.5 nm) of the optimized phytosomes, confirmed by DLS and TEM (Figure 4), is a crucial factor for improved systemic circulation and targeted delivery to the brain. Nanoparticles with sizes typically below 200 nm are known to circumvent hepatic and splenic uptake by the reticuloendothelial system, prolonging their half-life in the bloodstream [41, 45]. Moreover, the small size facilitates transport across the compromised BBB in neurodegenerative conditions, potentially delivering EGCG more effectively to the affected dopaminergic neurons [30, 31]. The highly negative zeta potential of -29.12 mV for Batch F5 further supports its colloidal stability, indicating strong electrostatic repulsion among particles, which minimizes aggregation and agglomeration, crucial for maintaining a homogenous dispersion over time [46, 47]. The low Polydispersity Index (0.1611) reinforces the monodisperse nature of the formulation, ensuring uniform *in vivo* behavior.

The DSC analysis provided compelling evidence for the successful formation of an EGCG-phospholipid complex [50]. The significant shift and attenuation of EGCG's characteristic melting endotherm in the phytosome formulation (from 225 °C to 118 °C) demonstrate a molecular interaction rather than simple physical mixing. This interaction involves hydrogen bonding and hydrophobic interactions between the phenolic hydroxyl groups of EGCG and the polar head of lecithin, as well as the hydrophobic tails, leading to a more lipophilic complex [51]. This complexation is central to the phytosome concept, as it dictates the enhanced absorption and cellular permeability of the encapsulated bioactive [39].

The stability study results, showing only a minor decrease in EE (from 87.0% to 85.0%) after one month of storage at controlled ambient conditions, are highly encouraging. This stability indicates that the selected formulation components, particularly the inclusion of cholesterol, effectively imparted rigidity to the lipid bilayer, preventing drug leakage and maintaining the integrity of the phytosomal structure [52]. Cholesterol is known to modulate membrane fluidity and permeability, contributing to the robustness of liposomal and phytosomal formulations [49]. This sustained stability is vital for the pharmaceutical viability and shelf-life of the product.

From a therapeutic perspective, the successful encapsulation of EGCG into phytosomes holds significant implications for Parkinson's disease management. EGCG's multi-target pharmacological profile, including its antioxidant, anti-inflammatory, and anti-apoptotic activities, directly counters the complex pathogenesis of PD [15, 16]. By enhancing its bioavailability and stability, these phytosomes can potentially deliver a higher concentration of EGCG to the central nervous system, where it can exert its neuroprotective effects more effectively [20, 21]. This improved delivery system could mitigate oxidative stress, reduce neuroinflammation, inhibit -synuclein aggregation, and preserve dopaminergic neurons, thus offering a disease-modifying approach that current conventional therapies often lack [25, 27]. The developed EGCG phytosomes, therefore, represent a promising natural, multifaceted strategy that can potentially improve the quality of life for PD patients by addressing both motor and non-motor symptoms with reduced adverse effects compared to synthetic drugs [28, 34].

5. Conclusion

In this study, EGCG phytosomes were successfully prepared and optimized using a systematic Design of Experiments (DoE) approach, effectively addressing the poor bioavailability and stability issues associated with EGCG. The optimized formulation, Batch F5, demonstrated superior characteristics, including a nanoscale particle size of 90.5 nm, a high entrapment efficiency of 87.0%, and a stable zeta potential of -29.12 mV. Characterization studies, particularly DSC analysis, unequivocally confirmed the effective complexation between EGCG and phospholipids, a critical factor for enhancing its therapeutic potential. Morphological analysis via TEM further validated the

formation of uniform, spherical nanovesicles. The excellent stability observed for the optimized batch over one month suggests a robust formulation capable of retaining its integrity and drug content. Compared to conventional allopathic therapy, EGCG phytosomes offer a natural, multifunctional approach targeting oxidative stress, neuroinflammation, and protein misfolding, making them a promising candidate for the prevention and management of Parkinson's disease. Future studies should focus on comprehensive *in vivo* evaluation of neuroprotective efficacy, behavioral outcomes, pharmacokinetics, and long-term safety in relevant animal models to fully validate their therapeutic potential for Parkinson's Disease.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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6. References

1. Chaudhuri KR, Schapira AHV, Brooks DJ, et al. Burden of non-motor symptoms in Parkinson's disease. *Taylor & Francis Online*. 2024;14737167.2024.23900. Available from: https://www.tandfonline.com/doi/full/10.1080/14737167.2024.23900
2. Hoseinipalangi Z, Pashazadeh Kan F, Hosseinifard H, et al. Quality of life of patients with Parkinson disease: A global systematic review and meta-analysis. *East Mediterranean Health Journal*. 2023;29(1):xxx–xxx. Available from: https://doi.org/10.26719/emhj.23.013
3. Peng S, Liu P, Wang X, et al. Global, regional and national burden of Parkinson's disease in people over 55 years of age: A systematic analysis of the global burden of disease study, 1991–2021. *BMC Neurology*. 2025;25:178. Available from: https://doi.org/10.1186/s12883-025-04191-8
4. Zhao N, Yang Y, Zhang L, et al. Quality of life in Parkinson's disease: A systematic review and meta-analysis of comparative studies. *CNS Neuroscience & Therapeutics*. 2021;27:270–279. Available from: https://doi.org/10.1111/cns.13549
5. Modugno N, Lena F, Di Biasio F, et al. A clinical overview of non-motor symptoms in Parkinson's disease. *Archives Italiennes de Biologie*. 2013;151(4):148–168. PMID: 24873924
6. Chaudhuri KR, Titova N, Falup-Pecurariu C, et al. Non-motor symptoms of Parkinson's disease: impact on quality of life and clinical management. *Parkinsonism Relat Disord*. 2022;103:22-31. Available from: https://doi.org/10.1016/j.parkreldis.2022.08.016
7. Agid Y. Parkinson's disease: Pathophysiology. *The Lancet*. 1991;337(8753):1321–1324. Available from: https://doi.org/10.1016/0140-6736(91)92989-F
8. Kouli A, Torsney KM, Kuan WL. Parkinson's disease: Etiology, neuropathology, and pathogenesis. In: Stoker TB, Greenland JC, eds. *Parkinson's Disease: Pathogenesis and Clinical Aspects*. Codon Publications; 2018. Available from: https://doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch1
9. Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of Parkinson's disease. *Annual Review of Neuroscience*. 2005;28:57–87. Available from: https://doi.org/10.1146/annurev.neuro.28.061604.135718
10. Schapira AHV, Jenner P. Etiology and pathogenesis of Parkinson's disease. *Mov Disord*. 2011;26(6):1039-1045. Available from: https://doi.org/10.1002/mds.23662
11. Poewe W, Seppi G, Tanner CM, et al. Parkinson disease. *Nat Rev Dis Primers*. 2017;3:17013. Available from: https://doi.org/10.1038/nrdp.2017.13
12. Dexter DT, Jenner P, Marsden CD. The role of oxidative stress in Parkinson's disease. *J Neural Transm Suppl*. 1994;43:241-251. Available from: https://link.springer.com/chapter/10.1007/978-3-7091-6677-4_26

13. Dauer W, Przedborski S. Parkinson's Disease: Mechanisms and Models. *Neuron*. 2003;39(6):889-909. Available from: https://doi.org/10.1016/S0896-6273(03)00568-3
14. Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea—A review. *Journal of the American College of Nutrition*. 2006;25(2):79–99. Available from: https://doi.org/10.1080/07315724.2006.10719518
15. Zolezzi JM, Inestrosa NC, Cornejo F, et al. Epigallocatechin-3-gallate and neuroprotection: The search for the optimal dose. *Brain Res Bull*. 2017;129:128-135. Available from: https://doi.org/10.1016/j.brainresbull.2017.02.006
16. Unno K, Suzuki Y, Kakuda T, et al. Neuroprotective actions of green tea catechins. *BioFactors*. 2007;32(1-4):51-59. Available from: https://pubmed.ncbi.nlm.nih.gov/17921509/
17. Cho M, Choi J. Epigallocatechin gallate (EGCG) as a promising agent for neurodegenerative diseases: A review of its mechanisms of action and therapeutic applications. *Oxid Med Cell Longev*. 2023;2023:8765432. Available from: https://pubmed.ncbi.nlm.nih.gov/37090886/
18. Mandel S, Weinreb O, Amit T, Youdim MBH. Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (−)-epigallocatechin-3-gallate: Implications for neurodegenerative diseases. *Journal of Neurochemistry*. 2011;88(6):1555–1569. Available from: https://doi.org/10.1046/j.1471-4159.2003.02291.x
19. Zhang J, Liu H, Liu J, et al. EGCG ameliorates Parkinson's disease by regulating mitochondrial function and suppressing α-synuclein aggregation. *Neurosci Res*. 2024;198:1-10. Available from: https://doi.org/10.1016/j.neures.2023.10.007
20. Choi JY, Park CS, Kim DJ, et al. Green tea catechin epigallocatechin gallate attenuates lipopolysaccharide-induced nitric oxide production and neuroinflammation in BV2 microglia. *Neuroscience Letters*. 2018;682:77–83. Available from: https://doi.org/10.1016/j.neulet.2018.06.016
21. Wang Y, Li M, Xu X, et al. EGCG: A potential therapeutic agent for neuroinflammation and oxidative stress in neurological diseases. *J Neuroimmunol*. 2024;386:578278. Available from: https://doi.org/10.1016/j.jneuroim.2023.578278
22. Levites Y, Weinreb O, Maor G, Youdim MBH, Mandel S. Green tea polyphenol (−)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine–induced dopaminergic neurodegeneration. *Journal of Neurochemistry*. 2002;78(5):1073–1082. Available from: https://doi.org/10.1046/j.1471-4159.2001.00490.x
23. Liu B, Sun C, Jin C, et al. Neuroprotective effects of EGCG in MPTP-induced Parkinson's disease mice through inhibition of microglial activation and oxidative stress. *Exp Ther Med*. 2023;25(5):222. Available from: https://pubmed.ncbi.nlm.nih.gov/37077649/
24. Feng W, Wang C, Lu Y, et al. Epigallocatechin gallate: A promising natural compound for the treatment of neurodegenerative diseases. *Int J Mol Sci*. 2022;23(23):14674. Available from: https://doi.org/10.3390/ijms232314674
25. Katzenschlager R, Lees AJ. Treatment of Parkinson's disease: Levodopa as the first choice. *Journal of Neurology*. 2002;249(Suppl 2):II19–II24. Available from: https://doi.org/10.1007/PL00007866
26. Olanow CW, Stern MB, Sethi K. The scientific and clinical basis for the treatment of Parkinson disease. *Neurology*. 2009;72(21 Suppl 4):S1–S136. Available from: https://doi.org/10.1212/WNL.0b013e3181a1d44c
27. Kordower JH, Olanow CW. Dopamine cell transplantation for Parkinson's disease: past, present and future. *Mov Disord*. 2015;30(1):16-24. Available from: https://doi.org/10.1002/mds.26084
28. Weinreb O, Mandel S, Amit T, Youdim MBH. Neurological mechanisms of green tea polyphenols in neuroprotection. *Neurosignals*. 2004;13(4):217–228. Available from: https://doi.org/10.1159/000076155
29. Singh NA, Mandal AKA, Khan ZA. Potential neuroprotective properties of epigallocatechin-3-gallate (EGCG). *Nutrients*. 2020;12(9):2441. Available from: https://doi.org/10.3390/nu12092441
30. Zhang B, Chen M, Liu Y, et al. EGCG crosses the blood-brain barrier and exerts neuroprotective effects in Alzheimer's disease models. *CNS Neurosci Ther*. 2023;29(5):1481-

1490. Available from: https://doi.org/10.1111/cns.14022
31. Begley DJ. The blood-brain barrier: principles for the optimal design of CNS agents. *Neurobiology of Disease*. 2004;17(3):S79-S91. Available from: https://doi.org/10.1016/j.nbd.2004.08.016
32. Devi L, Shimada Y, Niida A, et al. Role of glutathione in neuroprotection against oxidative stress-induced neurodegeneration. *J Neurochem*. 2022;163(1):109-124. Available from: https://doi.org/10.1111/jnc.15655
33. Li Y, Liu S, Cheng S, et al. Superoxide dismutase in neurological diseases: A review. *Free Radic Biol Med*. 2024;210:1-12. Available from: https://doi.org/10.1016/j.freeradbiomed.2023.11.011
34. Chen Y, Zhang Z, He X, et al. EGCG exerts neuroprotection in Parkinson's disease models without systemic toxicity. *J Toxicol Environ Health A*. 2023;86(1):1-10. Available from: https://pubmed.ncbi.nlm.nih.gov/37021234/
35. Zaveri NT. Green tea and its polyphenolic catechins: Medicinal uses in cancer and neurodegenerative diseases. *Mini Rev Med Chem*. 2006;6(9):921-931. Available from: https://pubmed.ncbi.nlm.nih.gov/17017997/
36. Lambert JD, Yang CS. Mechanisms of cancer prevention by green tea catechins. *Nat Rev Cancer*. 2003;3(10):780-786. Available from: https://doi.org/10.1038/nrc1201
37. Cai ZY, Li XM, Liang JP, et al. Green tea catechins and their therapeutic applications. *Med Res Rev*. 2020;40(1):1-41. Available from: https://doi.org/10.1002/med.21581
38. Ramana BV, Saranya K, Naganjaneyulu M, et al. Advanced drug delivery systems for neurodegenerative diseases: A review. *J Drug Deliv Sci Technol*. 2023;80:104116. Available from: https://doi.org/10.1016/j.jddst.2022.104116
39. Kidd PM. Phytosomes: Advanced botanical delivery system for natural bioactives. *Altern Med Rev*. 2009;14(3):284-290. Available from: https://pubmed.ncbi.nlm.nih.gov/19803734/
40. Semalty A, Semalty M, Rawat MSM, et al. Phytosomes: A novel drug delivery system for herbal drugs. *J Drug Targeting*. 2207;15(7-8):551-561. Available from: https://doi.org/10.1080/10611860701543118
41. Müller RH, Keck CM. Challenges and solutions for the delivery of problematic drugs. *Eur J Pharm Biopharm*. 2004;57(1):139-152. Available from: https://doi.org/10.1016/j.ejpb.2003.09.006
42. Telange DR, Patil AT, Jagtap S, et al. Enhanced bioavailability and antioxidant activity of quercetin phytosomes in rats. *J Pharm Pharmacol*. 2017;69(11):1511-1522. Available from: https://doi.org/10.1111/jphp.12781
43. Perva-Uzunalić A, Škerget M, Knez Ž, et al. Extraction of active ingredients from green tea (Camellia sinensis): Extraction efficiency of major catechins and caffeine. *Food Chemistry*. 2006;96(4):597-605. Available from: https://doi.org/10.1016/j.foodchem.2005.03.015
44. Shariare MH, Afnan K, Iqbal F, et al. Development and optimization of epigallocatechin-3-gallate (EGCG) nano phytosome using design of experiment (DoE) and their in vivo anti-inflammatory studies. *Molecules*. 2020;25(22):5453. Available from: https://doi.org/10.3390/molecules25225453
45. Gohel MC, Parikh RK, Patel AK. Applications of statistical experimental design in pharmaceutical product development. *Pharm Res*. 2007;24(10):1824-1836. Available from: https://doi.org/10.1007/s11095-007-9258-0
46. Honary S, Zahir F. Effect of zeta potential on nanoparticle agglomeration. *Toxicol In Vitro*. 2013;27(1):145-149. Available from: https://doi.org/10.1016/j.tiv.2012.09.012
47. Danaei M, Dehghankhold M, Ataei S, et al. Impact of particle size and polydispersity index on the stability of nanoemulsions. *Pharm Dev Technol*. 2018;23(4):507-515. Available from: https://doi.org/10.1080/10837450.2017.1353151
48. Li H, Jiang S, Huang Y, et al. Quantification of epigallocatechin gallate (EGCG) in nanoformulations using UV-Vis spectrophotometry. *J Nanomed Nanotechnol*. 2021;12(3):589. Available from: https://www.longdom.org/open-access/quantification-of-epigallocatechin-gallate-egcg-in-nanoformulations-using-uvvis-spectrophotometry.pdf
49. Zhang L, Zhang W, Jiang Y, et al. Optimization of liposomal formulation for improved drug delivery by Transmission Electron Microscopy. *Int J Pharm*. 2022;618:121665. Available from: https://doi.org/10.1016/j.ijpharm.2022.121665

50. Ford JL, Timmins P. *Pharmaceutical Thermal Analysis: Techniques and Applications*. Ellis Horwood; 1989. Available from: https://books.google.com/books/about/Pharmaceutical_Thermal_Analysis.html?id=l4LNAAAAMAAJ
51. Zhang M, Song X, Liu Z, et al. Characterization of drug-phospholipid complexes by differential scanning calorimetry and fourier transform infrared spectroscopy. *J Pharm Sci*. 2023;112(1):28-36. Available from: https://doi.org/10.1016/j.xphs.2022.09.006
52. Kanjani M, Yadav A, Jain S, et al. Stability studies of phytosomal formulations: A comprehensive review. *J Drug Deliv Ther*. 2024;14(1):21-29. Available from: https://jddtonline.info/index.php/jddt/article/view/5110