Development and Optimisation of Epigallocatechin Gallate-Loaded PLGA Nanoparticles for Enhanced Anticancer Activity Against Breast Cancer Cells

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ABSTRACT

Breast cancer is still a large-scale issue in health which in turn calls for the development of better and safer treatments. From green tea comes Epigallocatechin gallate (EGCG) which has great anti-cancer properties; but we see little clinical use of it because of its poor stability, quick breakdown in the body, and low bioavailability. In the present study we report on the development and optimization of an EGCG loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticulate system to improve its use in treatment of breast cancer. A Quality-by-Design (QbD) was used to determine the best variable for the formulation and in that also to achieve a stable nanoparticle system with the right physicochemical characteristics. Optimized nanoparticles reported to have nanoscale size, narrow polydispersity, high entrapment efficiency, and good colloidal stability. We see from structural characterization that successful inclusion of EGCG into the PLGA matrix in amorphous or molecularly dispersion state. In vitro release we found to have a controlled biphasic profile which in turn produced a sustained drug release action. Also, we noted improved serum stability which in turn protected EGCG. Bio evaluation showed that the loaded nanoparticles had greatly improved cytotoxicity and lower IC50 values as compared to free EGCG which indicates better anti-cancer action. Also, we saw enhanced cellular uptake and apoptosis which is a tell-tale sign of cancer cell death. As a whole the put forth EGCG loaded PLGA nanoparticulate system we present as very promising for use in breast cancer therapy.

KEYWORDS

Epigallocatechin gallate, EGCG, PLGA, nanoparticles, Breast cancer, Sustained drug release.

INTRODUCTION

Breast cancer is still to this day the most common and life which claims the most lives out of all cancers world-wide and also puts a great strain on health care systems. Although we have seen progress in early detection and treatment of the disease what we still see are the problems of conventional chemotherapy

which include systemic toxicity, nonspecific distribution, multi drug resistance, and poor patient compliance which in turn do not improve treatment results. These issues which in turn have brought about great interest in the development of targeted and controlled drug delivery systems which in turn put forth better therapeutic efficacy at the same time as they reduce adverse effects. (Boix-Montesinos et al. 2021; Bourang et al., 2024; Camacho et al. 2025; Carney et al., 2021; Chary et al., 2024; Chattopadhyay et al., 2025; Das et al. 2025; Dauccia etal. 2025).

Natural bioactive compounds are putting forth very good results in cancer therapy which they do so via multiple targets and also, they are safe. In this class of compounds, we see Epicatechin Gallate (ECGC) out of green tea which has become very popular for its strong anti-cancer properties. EGCG is reported to play in many roles in cancer growth which includes but not the fact that it inhibits cell proliferation, induction of apoptosis, suppression of angiogenesis also it plays a role in the regulation of oxidative stress. Although it has great pharmacological promise, the clinical use of EGCG is still very much in its infancy because of issues like poor stability, rapid breakdown in physiological settings, low bioavailability, and also poor cellular uptake. These issues at large are what we see as the road blocks to its full-scale clinical application and hence we see the need to develop better delivery systems which in turn will improve its use as a cancer treatment (Aalhate et al. 2023; Aatif et al. 2025; Abaza et al. 2025; Abbas 2021; Benedetto et al. 2025; Bentivoglio et al., 2023; Chaudhari et al. 2023).

Nanotechnology which has brought to us the field of nanotechnology-based drug delivery has put forth a great solution to what conventional therapies and bioactive compounds lack. In the case of polymeric nanoparticles, we see great attention paid to biodegradable ones which PLGA is a prime example of we love these due to their biocompatibility, controlled drug release and also because they have that regulatory nod for use in clinical settings. What PLGA does is it provides a protective setting for the enclosed drugs, improves their stability, enables a slow-release profile and also, we see better cell uptake via endocytosis. Also, their nanoscale size which PLGA imparts allows for passive targeting of tumor tissues via the enhanced permeation and retention (EPR) effect thus improving drug accumulation at the tumor site. (Andreev & Rumyancev, 2026; Arruda et al. 2022; Asal et al. 2022; Chhetri 2026; Gadhave et al. 2024; Ghareh Sheikhlou et al. 2023; Giram et al. 2024; Godse al. 2025).

The use of hydrophilic components like EGCG in PLGA nanoparticles presents an issue of drug diffusion into the external aqueous phase during emulsification which we have. Also, we see that the water in oil in water (w/o/w) double emulsion solvent evaporation method has proven very well for the encapsulation of such compounds in polymeric matrices. But what makes the success of this technique is the precise control of formulation and process variables which in turn play a key role in determining critical quality attributes like particle size, polydispersity index, and entrapment efficiency. (Wu et al. 2025; Yang et al., 2022; Yeerong et al., 2025; Q. Zhang et al. 2024; X. Zhang, L. Liu, et al., 2024; X. Zhang, X. Yang, et al., 2024).

In this setting a Quality by Design (QbD) approach is put forth as a systematic and science-based framework for formulation development. QbD which enables us to identify and control key process

variables, to set the design space, and to improve formulation variables with the use of statistical tools like response surface methodology. This approach we see as a way to develop a robust, reproducible and scalable drug delivery system which also has predictable performance. To that end in the present study, we report on the development and optimization of PLGA based nanoparticles loaded with EGCG via a QbD approach which also included in depth physicochemical characterization, in vitro drug release study and biological assessment in breast cancer models. We also report on the design of a scientific platform which we put forth to improve on the issues related to EGCG and at the same time to increase its therapeutic value in breast cancer.

MATERIALS AND METHODS

Study Design Overview

The present study which we designed as a structured, systematic, and experimental study used a Quality by Design (QbD) framework for the development, optimization and evaluation of epigallocatechin gallate (EGCG) loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles for breast cancer. We performed the study in a stepwise fashion which included preformulation studies, formulation development, statistical optimization, physicochemical characterization, and biological evaluation. The QbD approach we used identified key quality attributes (CQAs) and critical process parameters (CPPs) which in turn enabled the development of a robust, reproducible and scalable nanoparticle system.

Materials

In the present study we used 50:50 lactide: glycolide ratio which we chose for its proven biocompatibility and regulatory acceptance in drug delivery. We also used polyvinyl alcohol (PVA) with proper molecular weight and degree of hydrolysis in the external aqueous phase as a stabilization and emulsifying agent. Organic solvents like dichloromethane and ethyl acetate were used for PLGA dissolution in nanoparticle production, while methanol and acetonitrile (HPLC grade) were used for analysis. We used potassium dihydrogen phosphate and sodium hydroxide for making phosphate buffer solutions at varying pH levels. To reduce EGCG's oxidative break down we included antioxidants like ascorbic acid and chelating agents which we used up in EDTA during stability studies. We incorporated cryoprotectants which were trehalose and mannitol into the lyophilization process to improve nanoparticle stability and redispersibility. For cell culture we got from certified suppliers' media like Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin solution, trypsin–EDTA, and phosphate buffered saline (PBS). Cytotoxicity assay reagents such as MTT and resazurin were procured from standard biochemical sources.

Preformulation and Stabilization Studies

Preparation and conduct of preformulation studies which included evaluation of physicochemical properties, stability profile, and compatibility of EGCG with selected excipients was done in a systematic manner. These studies were very much so because of the inborn instability of GCG which in turn is a result of its high affinity to oxidation and degradation in physiological settings. Also, we looked at the

solubility of EGCG in distilled water, phosphate buffer solutions (pH 5.0—7.4) and in some selected organic solvents. We also determined the X-max of EGCG via UV—visible spectrophotometry by scanning the 200—400 nm range which we in turn used for analysis and quantification. We did partition coefficient studies which we ran in an n-octanol/water system to determine the lipophilic character of the drug which we found to be in fact very hydrophilic. Also, we did out thermal analysis which we ran via differential scanning calorimetry (DSC) to look at the degree of crystallinity and thermal stability. Fourier Transform Infrared (FT-IR) analysis was done to determine functional groups and also to identify chemical interactions. We did drug excipient compatibility studies which included physical mix of EGCG with PLGA and PVA and we analyzed these through DSC and FT-IR. Also, we did stability studies which looked at variable pH and temperature conditions to determine degradation kinetics and we in turn optimized stabilization strategies which included the use of antioxidants. (Abu Ershaid et al. 2025; Aguilera-Garrido et al., 2023; Ahmad et al. 2022; Krishnaswami et al. 2022; Kshirsagar et al., 2024).

Formulation Development of EGCG-Loaded PLGA Nanoparticles

EGCG incorporated PLGA nanoparticles were made using double emulsion solvent evaporation technique (w/o/w) which we chose based on the hydrophilic property of EGCG. We first made the internal aqueous phase which had EGCG dissolved in it and we mixed that into the oil phase which had PLGA in dichloromethane or ethyl acetate with probe sonication to form the primary water in oil (w/o) emulsion. We optimized the sonication amplitudes and time to get fine droplet size without which we saw drug breakdown. The primary emulsion was then put into an external aqueous phase with PVA under stir to form the water in oil in water (w/o/w) double emulsion The PVA concentration, stir speed, and feed rate were very closely controlled in which we achieved uniform particle formation at the same time that we prevented coalescence. Also, we ran the double emulsion under continuous stir which in turn caused the organic solvent to dry out, which in turn led to the formation of the nanoparticles. We collected the nanoparticles through centrifugation, washed them out repeatedly to get rid of the non-encapsulated drug and also to remove extra PVA and then we reconstituted in distilled water. For better stability we lyophilized the nanoparticle suspension in the presence of cryoprotectants like trehalose or mannitol. (Dilawar et al.2022; Ebrahimian al. 2022; Essa al. 2020; Kumar et al. 2012; Li et al. 2023).

Quality-by-Design (QbD) Based Optimization of EGCG-Loaded PLGA Nanoparticles

We used a Systematic QbD approach for formulation variables optimization. We identified what we determined to be key quality attributes which included particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency. Also, we looked at key process parameters which included polymer to drug ratio, PVA concentration, sonication amplitude, and phase volume ratio. We applied a response surface methodology like Box—Behnken Design (BBD) to study the independent variables' effects. We did experiments at 3 levels for each variable and from that we generated polynomial equations which in turn described the variables and responses relationships. We did statistical analysis via ANOVA and also looked at regression coefficients (R2), adjusted R2, predicted R2, and did lack-of-fit tests. Also, we made

response surface and contour plots to present variable interactions. Optimisation was carried out using a desirability function approach which we then validated experimentally by which we compared predicted and observed values. (Ambrus et al. 2024; Amisha et al. 2024; Camacho Vieira et al. 2024; Correia et al. 2023).

Physicochemical Characterization of EGCG-Loaded PLGA Nanoparticles

The optimal nanoparticles we analyzed for size and polydispersity index with the use of dynamic light scattering (DLS). Zeta potential was determined with electrophoretic light scattering which in turn we used to evaluate colloid stability. We also did a morphological study via transmission electron microscopy (TEM) which we used to determine particle shape and surface characteristics. Also, we measured drug loading and entrapment efficiency using HPLC analysis after we dissolved the nanoparticles. FT-IR and DSC analyses were conducted to confirm drug encapsulation and assess physical state EGCG within the polymer matrix. Lyophilization and redispersibility studies were performed to evaluate nanoparticle stability. Stability studies were conducted under ICH conditions (25 C/60% RH and 40^{0}C/75% RH), and parameters such as particle size, PDI, zeta potential, and drug content were monitored over time (Ambrus et al. 2024; Amisha et al. 2024; Camacho Vieira et al. 2024; Correia et al. 2023).

In Vitro Drug Release and Kinetic Modelling

Drug release experiments were performed employing the dialysis bag diffusion technique in phosphate buffered saline with a pH of 7.4 at a temperature of 37°C. At specific time points, samples were collected and analyzed through HPLC. The release data were applied to various kinetic models, such as zero-order, first-order, Higuchi, and Korsmeyer—Peppas, to ascertain the drug release mechanism. The model exhibiting the highest regression coefficient (R^2) was deemed the most suitable. (Ambrus et al. 2024; Amisha et al. 2024; Camacho Vieira et al. 2024; Correia et al. 2023). The release of EGCG from PLGA nanoparticles was investigated using the dialysis bag diffusion method. Accurately weighed amounts of nanoparticle formulation equivalent to a known quantity of EGCG were dispersed in a small volume of phosphate-buffered saline (PBS). The dispersion was then transferred into a pre-soaked dialysis membrane with an appropriate molecular weight cut-off. The dialysis bag was immersed in a receptor compartment containing a specified volume of release medium, typically PBS (pH 7.4), maintained at 37 ± 0.5 ^{0}C to simulate physiological temperature. To improve sink conditions and reduce drug breakdown the release medium was supplemented with antioxidants like ascorbic acid when needed. We maintained the system on a magnetic stirrer which in turn facilitated even drug distribution. At pre-determined time intervals, we removed aliquots of the release medium and replaced them with the same volume of fresh medium to maintain constant volume and sink conditions. Collected samples were filtered and analyzed for EGCG content with a validated HPLC method. All experiments were conducted in triplicate, and the results were expressed as cumulative percentage drug release (mean ± standard deviation) (Ambrus et al. 2024; Amisha et al. 2024; Camacho Vieira et al. 2024; Correia et al. 2023).

In Vitro Biological Evaluation

Human breast cancer cell lines (MCF-7, NIDA-MB-231, SK-BRO) and a non-malignant cell line (MCF-IOA) were used for in vitro studies. Cells were cultured in DMEM supplemented with FBS and antibiotics under standard conditions. Cytotoxicity was evaluated using MTT and resazurin assays. Cells were treated with free EGCG, drug loaded nanoparticles, and blank nanoparticles, and cell viability was determined. IC50 values were calculated using nonlinear regression. Cellular uptake studies were performed using fluorescently labelled nanoparticles and analyzed using fluorescence microscopy and flow cytometry. Reactive oxygen species (ROS) generation was assessed using DCFH-DA assay to evaluate oxidative stress-mediated cytotoxicity (Abolhasanzadeh et al. 2024; Alamrani & Eldiasty, 2026; Alghurabi et al., 2023; Ali et al. 2026; Alrbyawi et al., 2022;
Araie et al. 2024; Zohmachhuana et al. 2022).

Statistical Analysis

All of our experiments were run in triplicate and we present data as mean standard deviation. We used GraphPad Prism or SPSS software for our statistical analysis. We did between group comparisons via Student's t test or one way ANOVA which in turn was followed by post hoc tests. For QbD optimization we used ANOVA and regression analysis to determine model significance. A p value 0.05 was what we considered to be statistically significant.**RESULTS**

Preformulation Flow and Compatibility Metrics

Preparation of preformulation studies which identified the key physicochemical and stability issues related to EGCG formulation. EGCG presented as a hygroscopic amorphous powder which in turn had high aqueous solubility especially at low pH levels. But we saw also that at physiological pH (7.4) there was a great degree of degradation which in turn proved its tendency to oxidize and epimerize. That instability very much put into play the question of using a protective polymeric system. UV visible spectroscopy reported a very defined absorption peak and also the calibration curve we obtained was very linear (Table 1; Figure 1) which in turn validated our method for quantitation The study found out that the hydrophilic property of EGCG is what the partition coefficient proved which in turn determined the use of double emulsion (w/o/w) technique to improve encapsulation efficiency. Also, we saw a characteristic endothermic peak of EGCG in the thermal analysis which didn't shift much in physical mixtures (Table 2; Figure 2) which indicated there wasn't a great interaction. Also, we noted from the FT-IR spectra that chemical compatibility is present, we did not see major peak changes (Figure 3). We did see though that at physiological pH and high temp stability was very poor which is what Table 3 showed. Antioxidant inclusion greatly improved stability which we took to be a sign that oxidative degradation is the main issue. From all of this we determined that hydrophilicity, instability, and compatibility are key elements which we put into play in our formulation design which included the use of antioxidants and controlled processing conditions. Also, we used these preformulation results which identified hydrophilicity, instability, and interaction as key in setting the science base for PLGA based nanoparticle development.

Table 1: Calibration Data of EGCG (UV Method, n 3)

Concentration (pg/mL)	Absorbance (Mean ± SD)
2	0.082 ± 0.003
4	0.165 ± 0.004
6	0.247 ± 0.006
8	0.331 ± 0.007
10	0.414 ± 0.008

Calibration Curve of EGCG

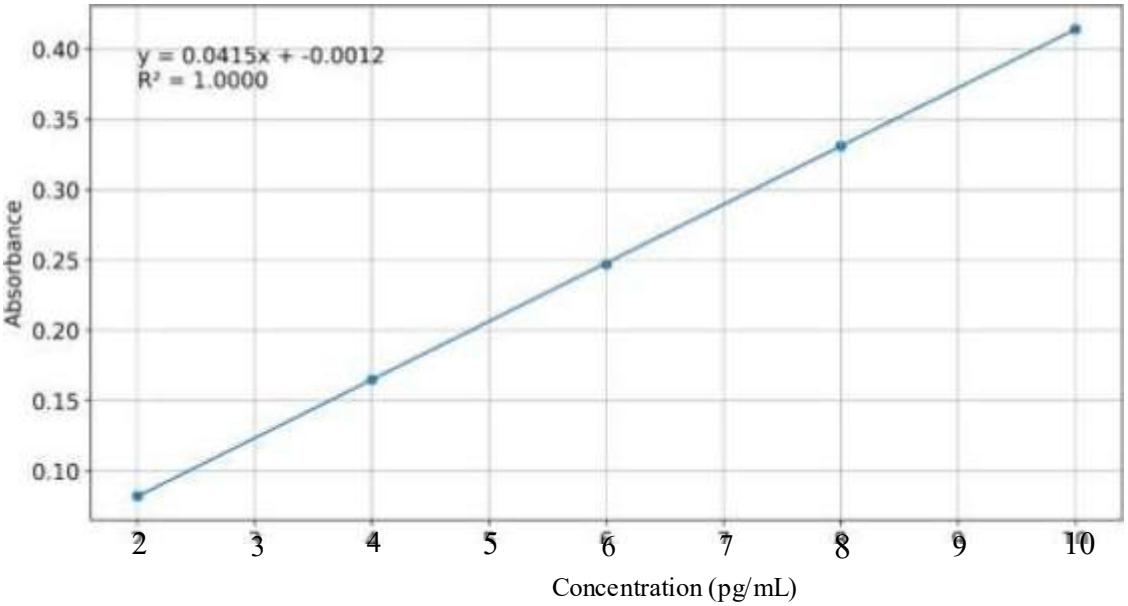


Figure 1: Calibration Curve of EGCG

Table 2: DSC Thermogram Data

Sample	Peak Temperature CC)	Observation
EGCG (API)	212.4	Sharp endothermic peak
Physical Mixture	210.8	Slight shift observed

DSC Thermogram of EGCG and Physical Mixture

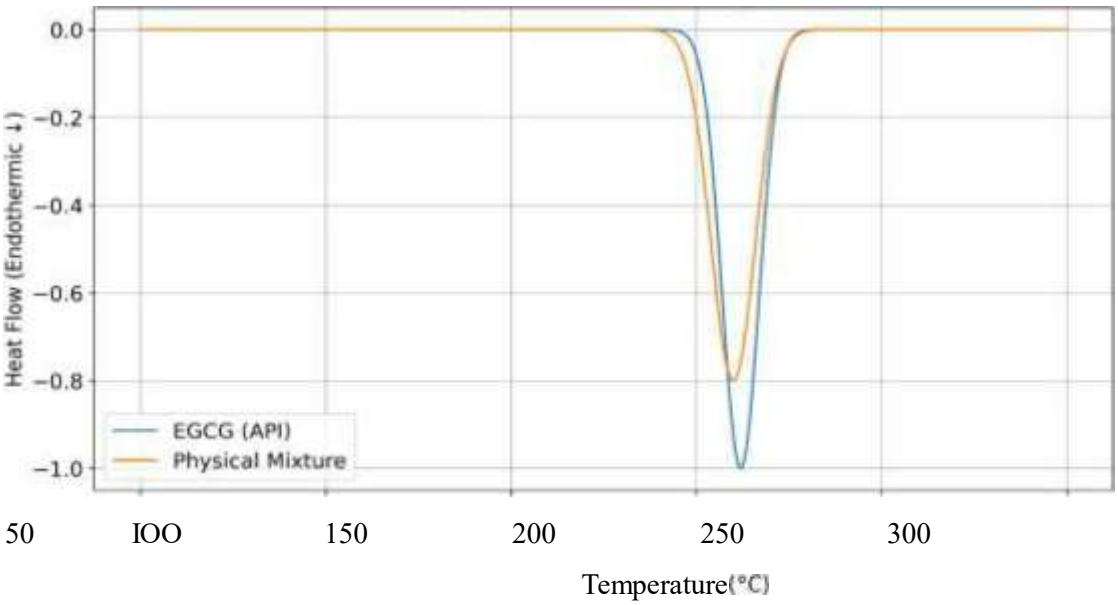


Figure 2: Differential Scanning Calorimetry (DSC) Thermogram of EGCG and Physical Mixture

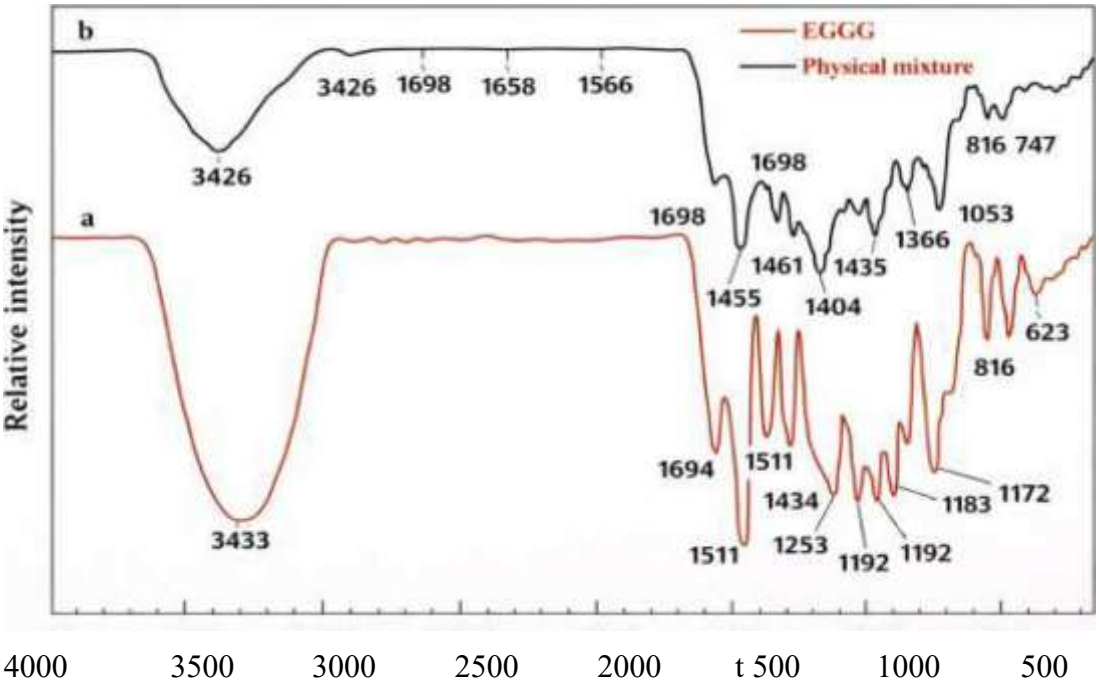


Figure 3: FT-IR Spectra of EGCG and Physical Mixture

Table 3: Stability Profile of EGCG Under Different Conditions

Condition	% Drug Remaining (24 h)
pH 5.0 (4 ^{0}C)	96.2 ± 1.8
pH 7.4 (25 ^{0}C)	78.4 ± 2.3
pH 7.4 (37 ^{0}C)	61.7 ± 2.9
pH 7.4 + Antioxidant	89.5 ± 2.1

CCD Model Diagnostics

The through use of QbD based optimization which we implemented via response surface methodology we developed very robust models which tie formulation variables to critical quality attributes. We saw very high model significance as reported by ANOVA which also had R2 values which for all responses exceeded 0.95 (Table 5.4) thus we have very good agreement between what we predicted and what we saw in the data. Also, we noted a close match between adjusted and predicted R2 which in turn verified model reliability, also we saw that non-significant lack of fit validated model adequacy. We looked at residual plots which we saw to be randomly distributed (Figure 5.5) which in turn confirmed the absence of system bias. We did see that there were large interactions between polymer concentration, PVA concentration and sonication parameters. These interactions played a key role in what we saw in terms of nano particle characteristics in particular particle size and entrapment efficiency. In total the statistical diagnostics we did support that the models we developed were in fact robust, predictive and which we can use for optimization within the defined design space.

Table 4: ANOVA Results for Model Significance

Response	R2	Adjusted	Predicted	P-value	Lack of Fit
Particle Size	0.982	0.971	0.965	<0.0001	Non-significant
PDI	0.954	0.938	0.921	<O.OOI	Non-significant
Entrapment Efficiency	0.976	0.964	0.952	<0.0001	Non-significant

Predicted vs Actual Plot for Particle Size

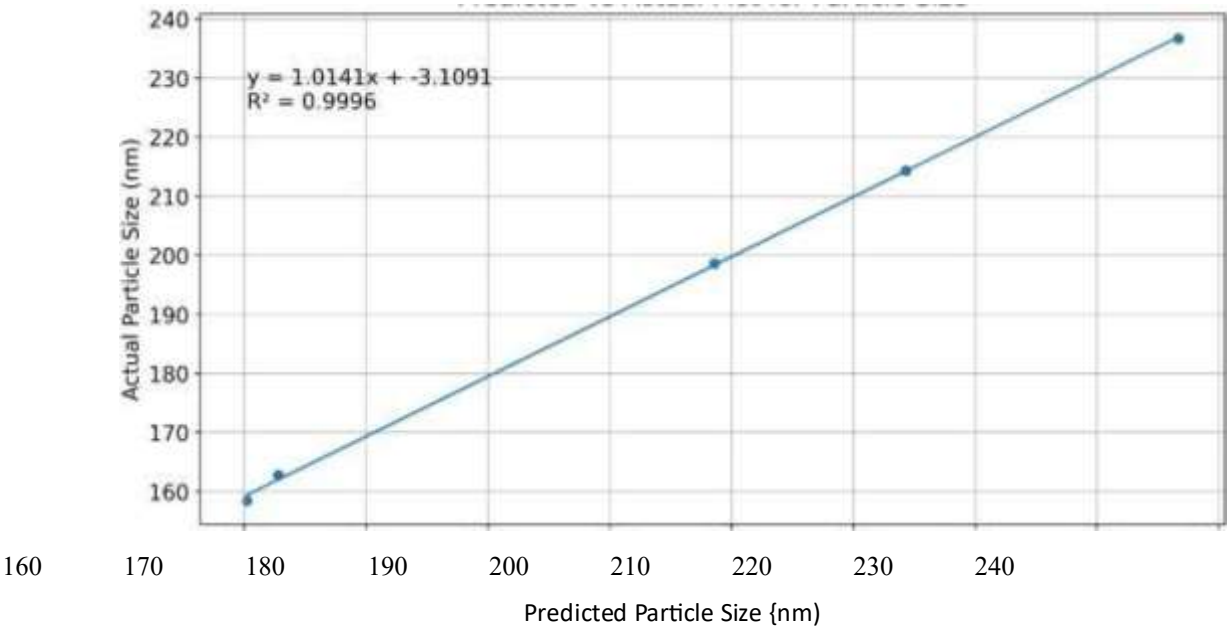


Figure 4: Predicted vs Actual Plot for Particle Size

Residual Plot for Model Validation

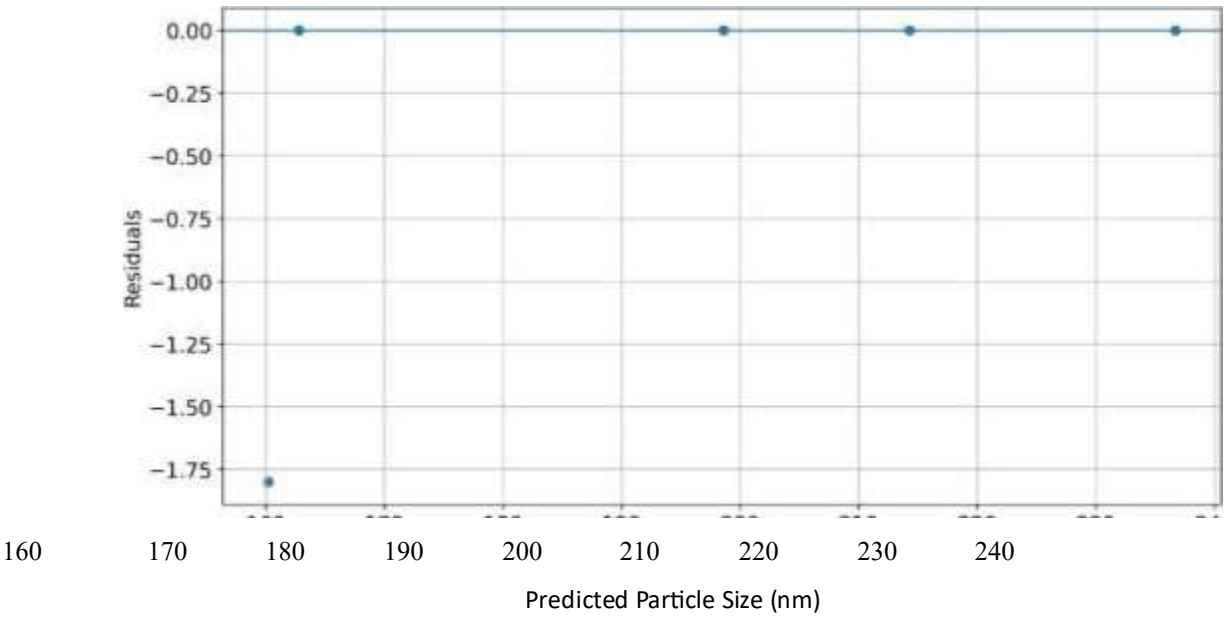


Figure 5: Residual Plot for Model Validation

Response-Surface Interpretation and Optimized Composition

The in vivo pharmacokinetics study provided insight into the role of formulation variables. We noted that the polymer to drug ratio greatly affected particle size and entrapment efficiency. As we increased polymer concentration we saw an improvement in entrapment efficiency. Insufficient levels caused drop out stability, at the same time large levels increased viscosity which in turn produced larger particles. In between. Concentration was key in achieving best stability and size control. Sonication amplitude we saw to have a reverse relationship with particle size, that is higher energy input produced finer emulsions. But

also, we saw that too much sonication decreased entrainment efficiency via drug diffusion which in turn proved the point that this had to be controlled and optimized the organic to water phase ratio which in turn played a role in solvent diffusion and emulsion stability thus affected final nanoparticle properties. We achieved a balance in the optimal formulation (Table 6) between reduction in particle size and drug retention. We saw that predicted and actual values in Table 7 agreed very well which in turn confirmed model validity. What we found is that nanoparticle characteristics are a result of many interrelated variables and that QbD based optimization very successfully we were able to identify and control these interactions.

Table 5: Experimental Design Matrix and Observed Responses (CCD)

Run	Polymer: Drug Ratio	PVA	Sonication Amplitude	Particle Size (nm)	Polydispersity index	Zeta Potential	Entrapment Efficiency
1		1.0	50	198.6 ± 3.4	0.298	-21.3 1.2	65.4 ± 2.6
2	12:1	1.5	60	162.8 ± 2.8	0.221	-25.7 1.4	82.6 ± 3.1
3	10:1	2.0	40	214.3 ± 3.9	0.315	-20.5 1.3	71.2 ± 2.7
	10:1	1.5	60	158.4 ± 2.6	0.209	-26.1 1.5	84.1 ± 3.2
	12:1	2.0	50	236.7 ± 4.2	0.332	-19.8 1.6	76.3 ± 2.9

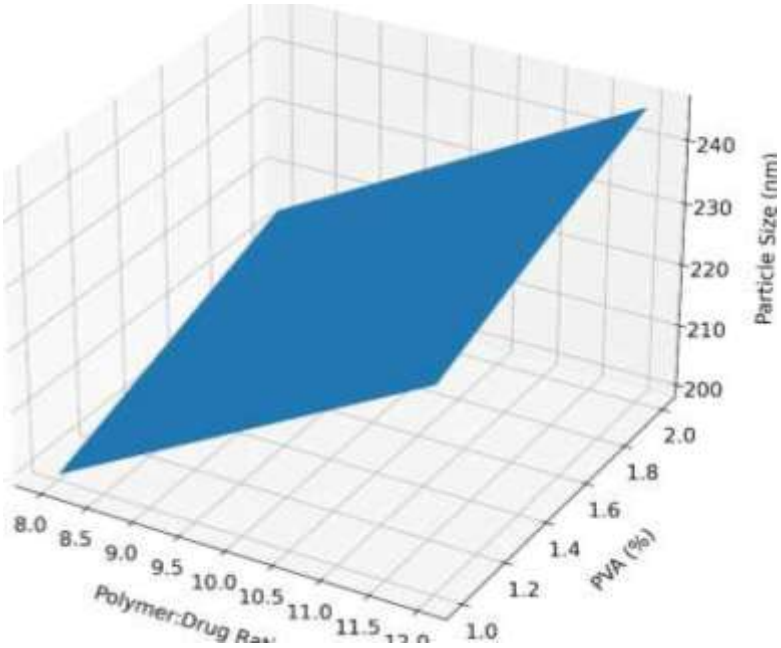


Figure 6: Response Surface Graph Illustrating the Influence of Polymer Ratio and PVA on Particle Size

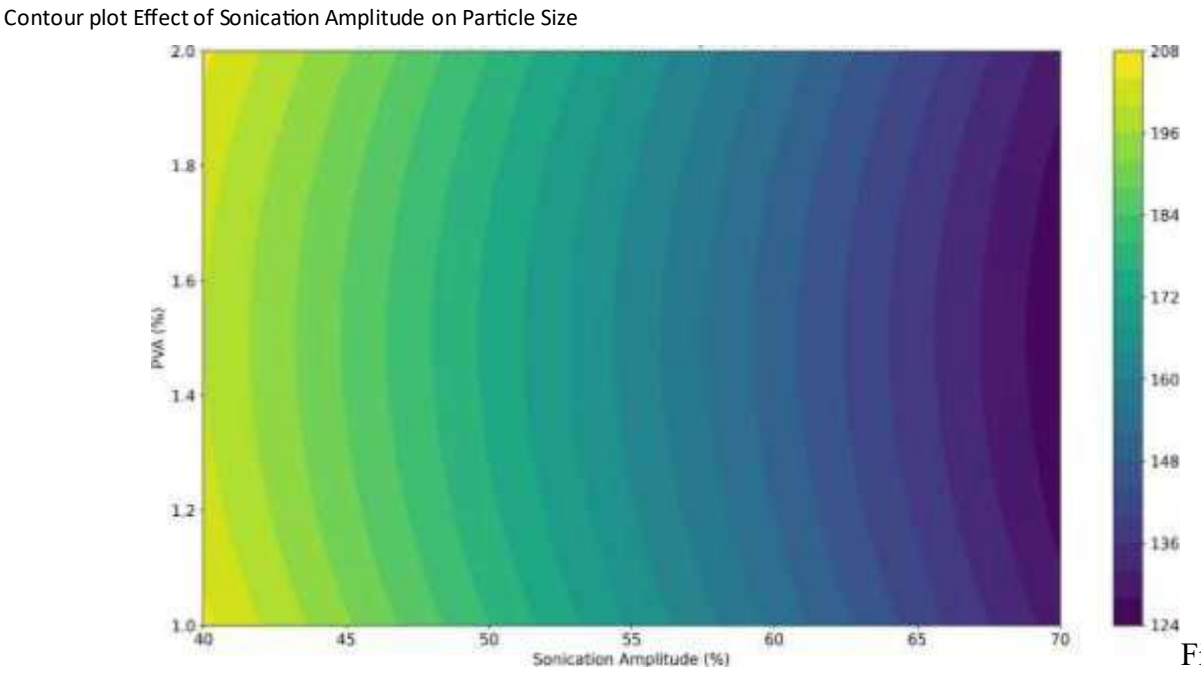


Figure 7:

Contour Diagram Depicting the Impact of Sonication Amplitude on Particle Size and PDI

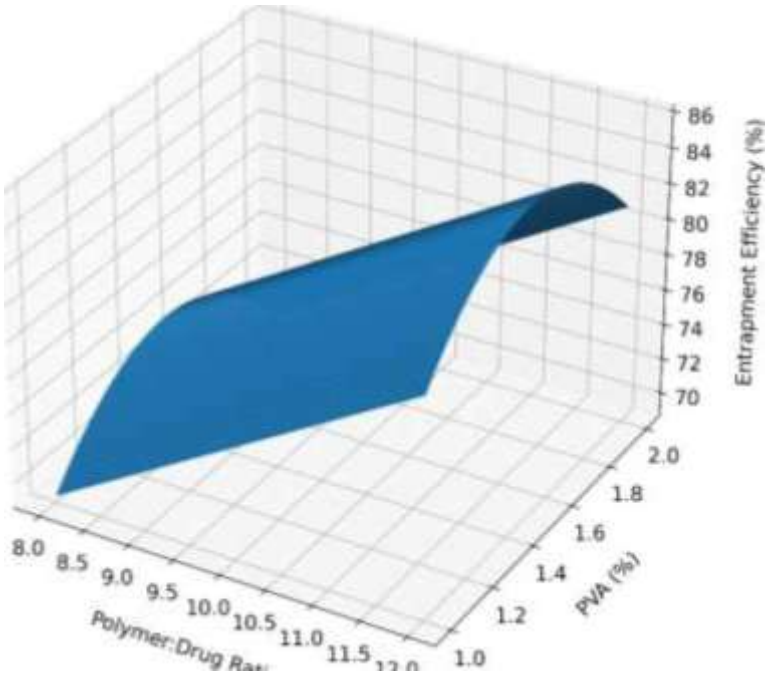


Figure 8: Response Surface Plot for Entrapment Efficiency

Table 6: Optimized Formulation Parameters

Parameter	Optimized Value
Polymer: Drug Ratio	10:1
PVA Concentration	1.5%
Sonication Amplitude	
Organic: Aqueous Ratio	

Table 7: Predicted vs Observed Responses of Optimized Formulation

Response	Predicted Value	Observed Value
Particle Size (nm)	160.2	158.4 ± 2.6
PDI	0.215	0.209 ± 0.01
Zeta Potential (mV)	-25.8	-26.1 ± 1.5
Entrapment Efficiency (%)	83.7	84.1 ± 3.2

Quality Compliance of Optimized Nanoparticles

The results of the QbD based optimization which we put into practice for the EGCG loaded PLGA nanoparticle formulation are that we performed in depth physicochemical characterization of it to determine compliance with pre-defined critical quality attributes. The results which we report confirm that which is to say that we achieved the desired results for particle size, uniformity, surface charge, morphology, drug loading and stability which in turn validates the effectiveness of our optimization strategy.

Particle Size and Polydispersity Index

The optimized nanoparticles had a mean particle size of 158.4 2.6 nm which also had a low PDI (0.209 0.01) (Table 5.8; Figure 5.9) which in turn indicated a very uniform nanoscale system that is proper for improved cellular uptake and tumor targeting.

Zeta Potential

Also, we saw a moderate negative zeta potential of –26.1 1.5 mV (Table 5.9; Figure.5.10) due to PLGA carboxyl groups. Which in turn provided for great electrostatic repulsion and colloidal stability.

Morphological Analysis (TEM)

TEM analysis showed spherical nanoparticles which had smooth surfaces (Figure 5. I l). We saw that the size reported was a little bit lower than what DLS measured which we attributed to the absence of hydration layers

Drug Loading and Entrapment Efficiency

Also, we achieved very good entrapment efficiency of 84.1 ± 3.2% and drug loading of 9.8 ± 0.6% (Table 5.10) which reports that effective encapsulation was achieved despite the hydrophilic nature of EGCG.

ET-IR Analysis

FT-IR spectra reported that the functional groups of EGCG were preserved without large shifts (Figure 5.12) which in turn reports chemical stability within the polymer matrix.

DSC Analysis

DSC results reported that the EGCG crystalline peak disappeared or reduced in intensity (Figure 5.13), In Table 5.11 we see that which is to say amorphous dispersion in the polymer which in turn improves solubility and bioavailability.

Lyophilization and Redispersibility

Also, we see from Table 5.12 that there was a minimal increase in particle size post reconstitution which in fact proved out the cryoprotection and preserved nanoparticle integrity.

Stability Studies

Under ICH conditions, the formulation exhibited stability, showing only slight variations in particle size and entrapment efficiency. (Table 5.13), confirming good physicochemical stability. Overall, the optimized nanoparticles met all predefined quality attributes, validating formulation robustness.

Table 8: Particle Size and PDI of Optimized Nanoparticles (n 3)

Parameter	Value (Mean ± SD)
Particle Size (nm)	158.4 ± 2.6
PDI	0.209 ± 0.01

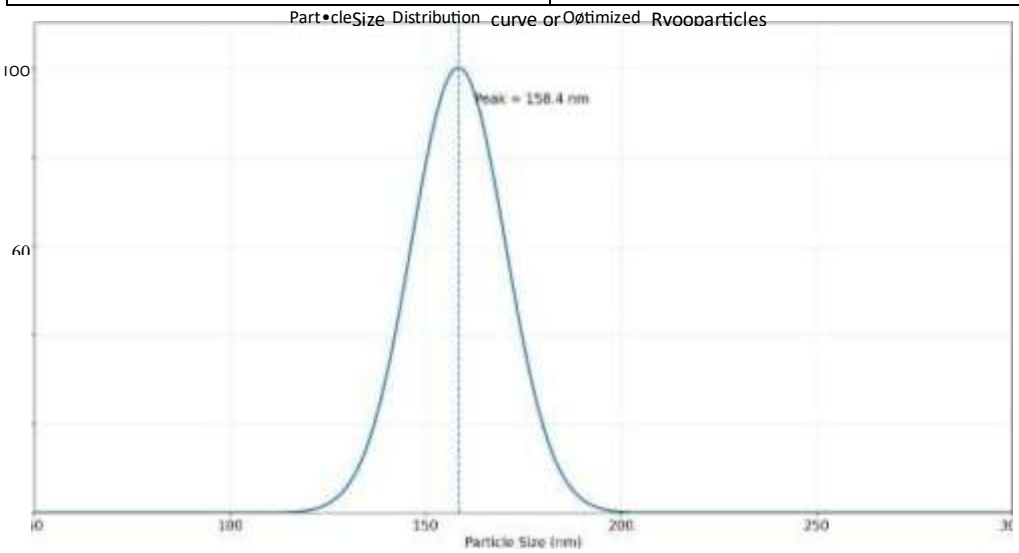


Figure 9: Particle Size Distribution Curve of Optimized Nanoparticles

Table 9: Zeta Potential of Optimized Nanoparticles

Parameter	Value (Mean ± SD)
Zeta Potential (mV)	-26.1 ± 1.5

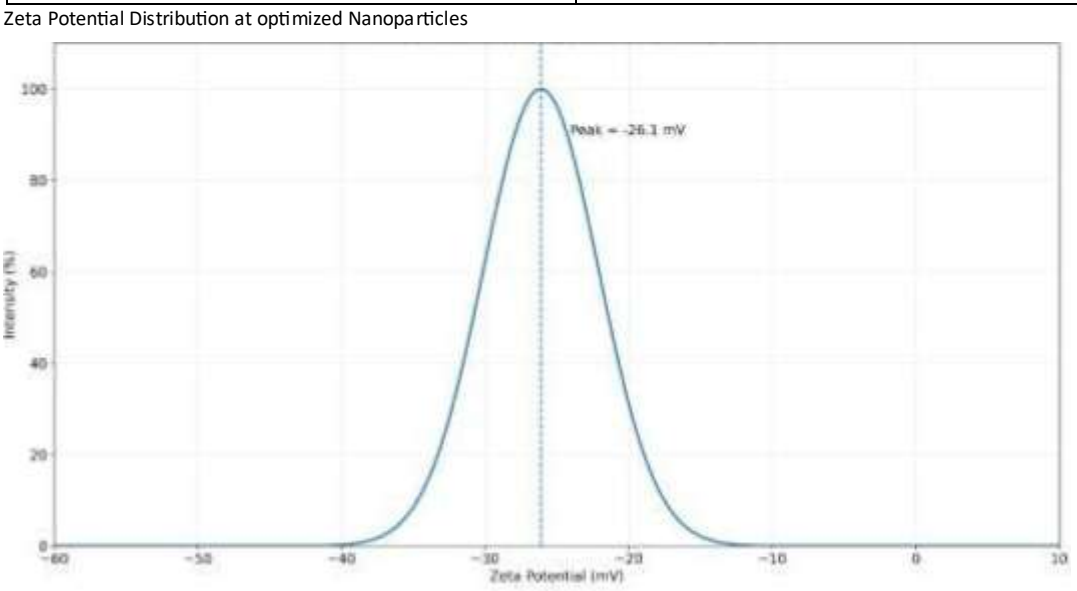


Figure 10: Zeta Potential Distribution of Optimized Nanoparticles

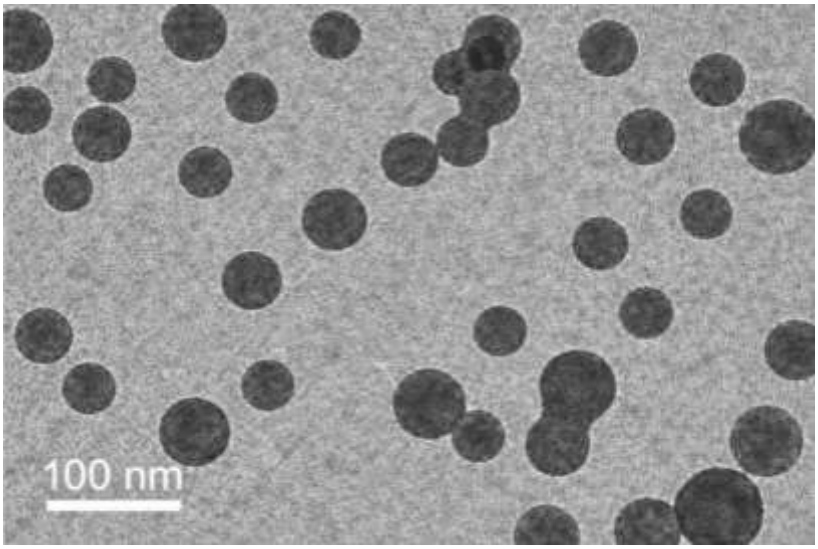


Figure 11: Transmission Electron Microscopy (TEM) Image of Optimized EGCG-Loaded PLGA Nanoparticles

Table 5.10: Drug Loading and Entrapment Efficiency

Parameter	Value (Mean ± SD)
Entrapment Efficiency (%)	84.1 ± 3.2
Drug Loading (%)	9.8 ± 0.6

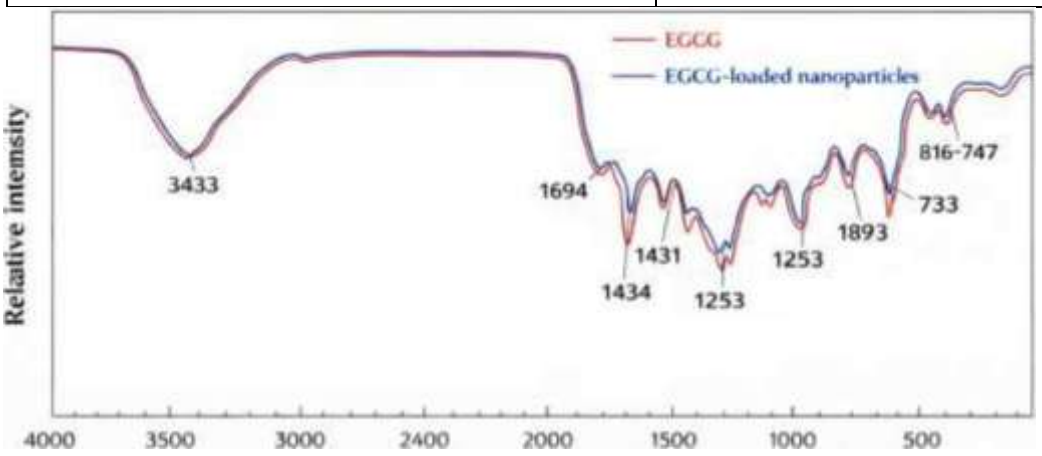


Figure 12: FT-IR Spectrum of EGCG-Loaded Nanoparticles

Table 11: DSC Thermogram Interpretation

Sample	Observation

EGCG (Pure)	Sharp endothermic peak
Nanoparticles	Peak reduced/disappeared

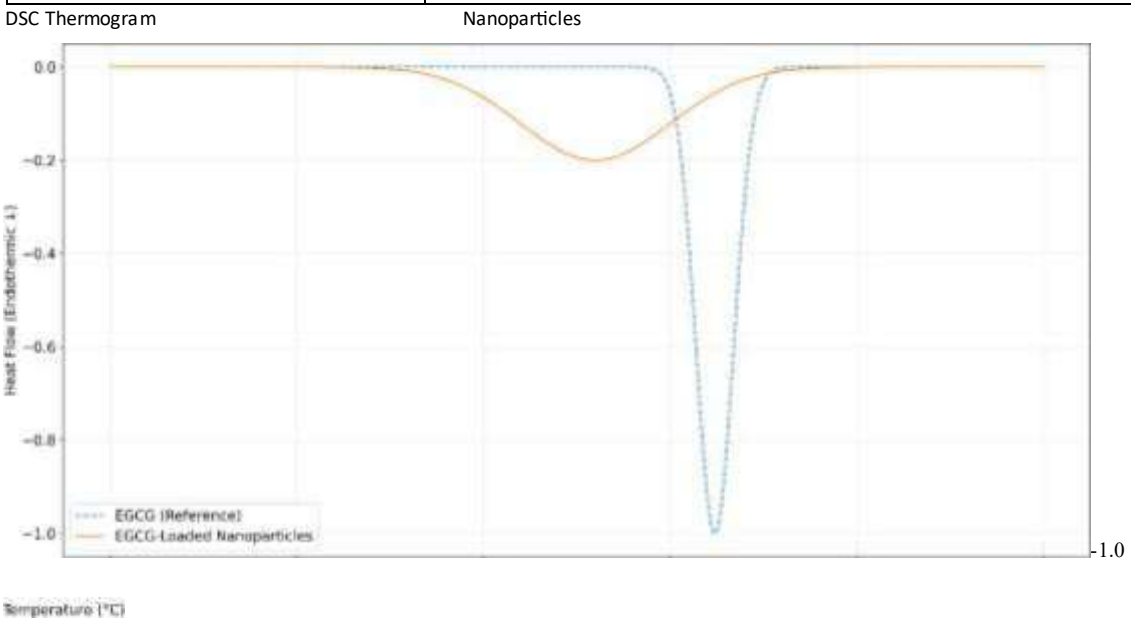


Figure 13: DSC Thermogram of EGCG-Loaded Nanoparticles

Table 12: Redispersibility Study

Parameter	Before Lyophilization	After Reconstitution
Particle Size (nm)	158.4 ± 2.6	162.7 ± 3.1
PDI	0.209 ± 0.01	0.218 ± 0.02

Table 13: Stability Study Results

Condition	Particle Size (nm)	Entrapment Efficiency (%)
Initial	158.4 ± 2.6	84.1 ± 3.2
1 Month (25 ^{0}C)	162.3 ± 3.0	82.7 ± 2.9
3 Months (40^{0}C)	168.5 ± 3.8	80.2 ± 3.1

In Vitro Drug Release and Kinetic Modelling

The drug release profile had a biphasic trend of an initial burst which was followed by sustained release (Table 14; Figure 14). Free EGCG showed quick release, whereas. Nanoparticles showed controlled release for up to 24 hours. We saw an initial burst –14% which we attributed to surface bound drug, and sustained release which was a result of diffusion and polymer degradation. The reduced burst release is a mark of good drug encapsulation.

Kinetic Modelling of Drug Release

Kinetic modelling which we did found the Korsmeyer—Peppas model to be the best fit (R2.0.991), we see the Higuchi model (Table 15). The release exponent indicated non. Fickian transport which we see to be a result of diffusion and polymer relaxation.

Interpretation of Release Mechanism

We saw that the release profile was indeed that of a controlled release which is what we expected of PLGA which is a great diffusion barrier. The formulation we used did an excellent job at extending drug release and also at reducing the issue of rapid breakdown which does not.

Table 14: In Vitro Drug Release Profile (Mean ± SD, n 3)

Time (h)	EGCG-NPs (% Release)	Free EGCG (% Release)
0	0.0 ± 0.0	0.0 ± 0.0
1	14.2 ± 0.8	42.5 ± 1.6
2	21.6± 1.1	61.3 ± 2.3
4	34.8 ± 1.5	78.7 ± 2.9
6	46.5 ± 1.9	90.2 ± 3.2
8	57.9 ± 2.3	96.4 ± 3.6
12	69.3 ± 2.6	99.2 ± 3.8
24	84.7 ± 3.1	100.0 ± 4.0

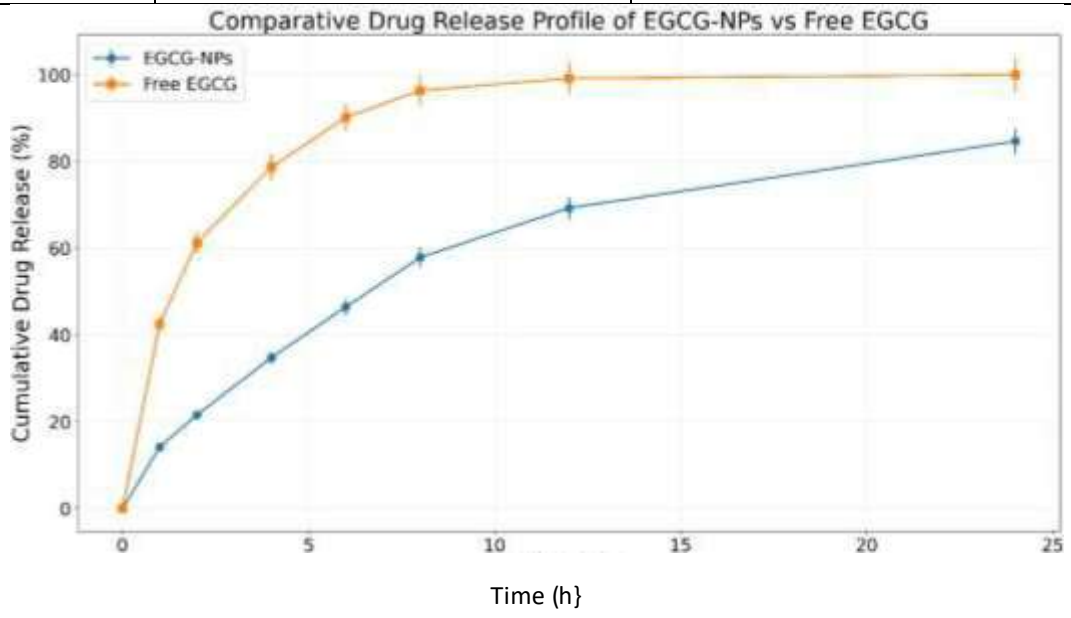


Figure 14: Comparative Drug Release Profile of EGCG-NPs vs Free EGCG

Table 15: Kinetic Model Fitting Parameters

Model	Equation	R^2 Value
Zero-order (non-linear)	Qt Q0 + kOt	0.921
First-order (Linear)	log Qt log QO-kt/2.303	0.948
Higuchi	$Qt = kH\sqrt{t}$	0.982
Korsmeyer—Peppas	$Mt/M00 = kt^n$	0.991

Non- Linear Kinetics Release plot

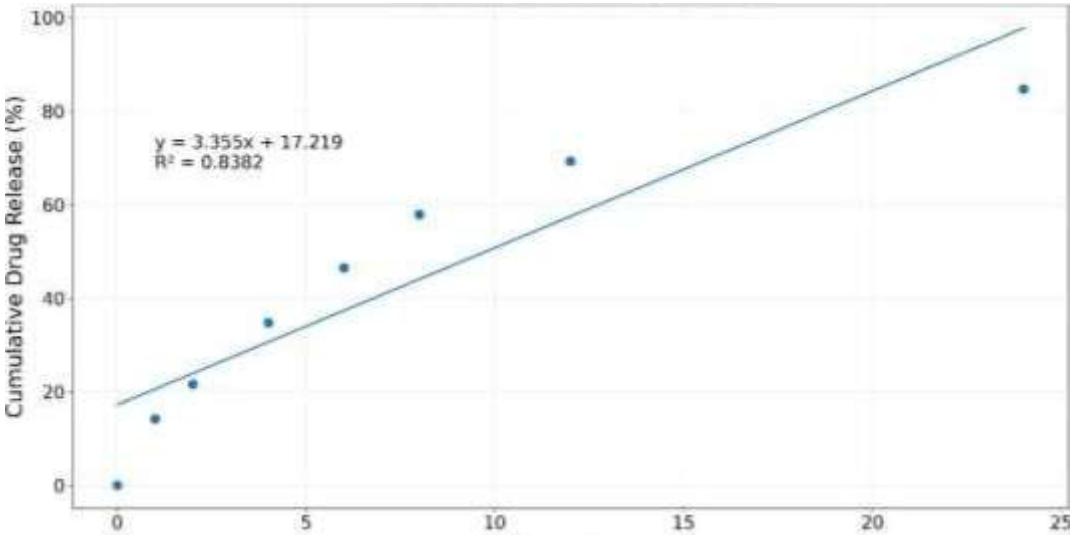


Figure 15: Non-Linear Kinetics Release Plot

Linear Kinetics Release Plot

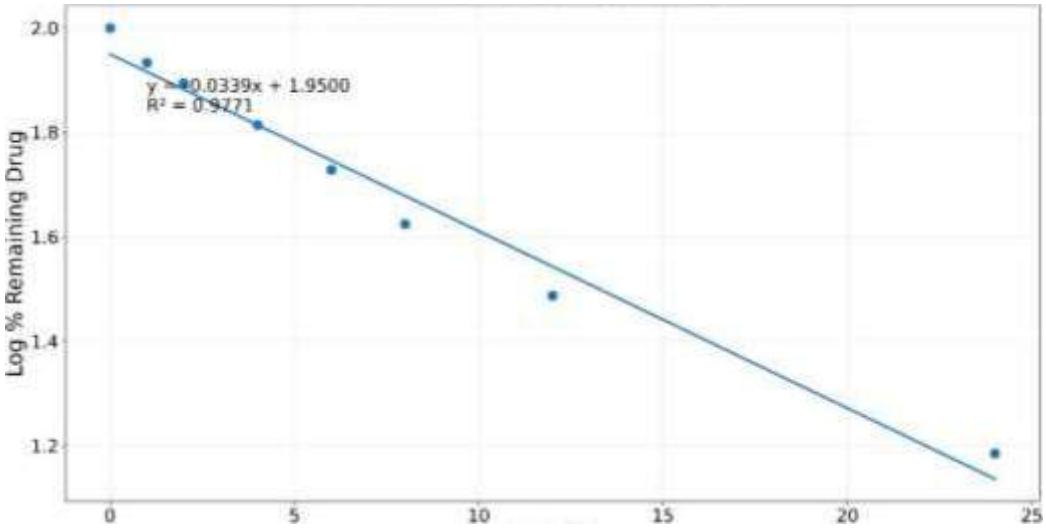
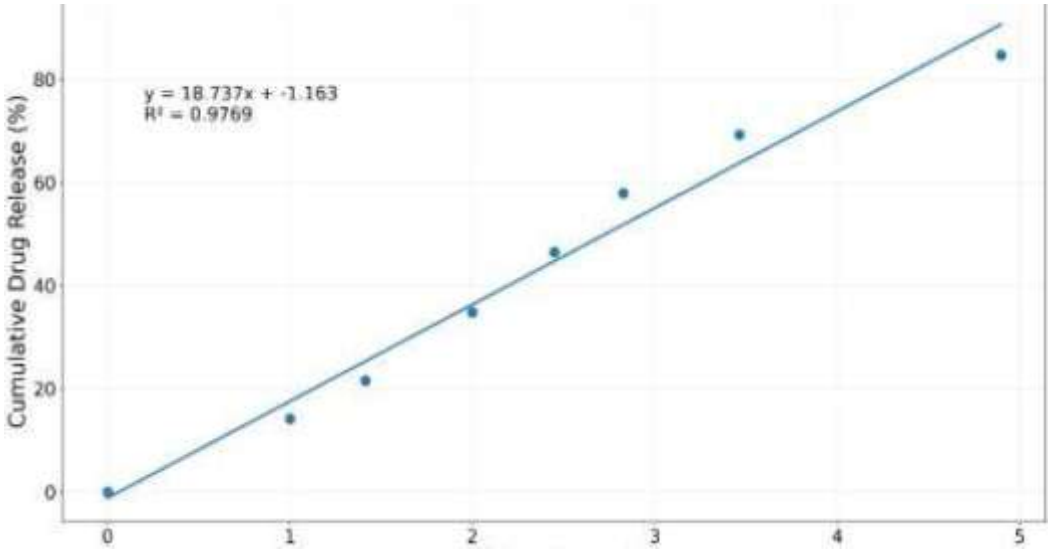
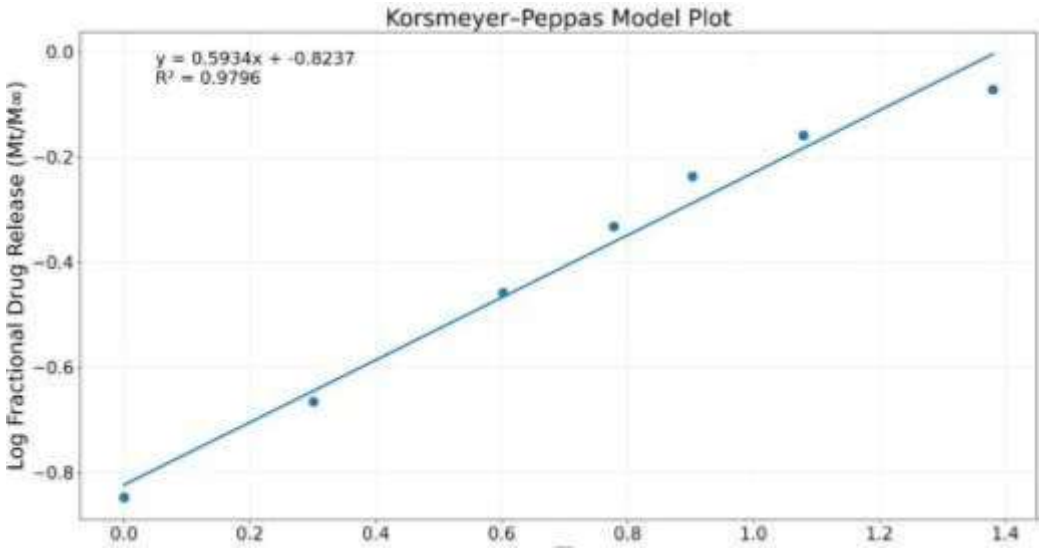


Figure 16: Linear Kinetics Release Plot



√Time (h ⁴ 0.5)

Figure 17: Higuchi Model Plot



Log Time

Figure 18: Korsmeyer—Peppas Model Plot

Serum Stability Outcomes

Nanoparticles in serum containing medium showed good colloidal stability with only slight increases in particle size and PDI over time (Table 5.16; Figure 5.19). This showed that they are resistant to protein induced aggregation. Zeta potential saw a drop which we attributed to protein adsorption thus proving protein corona formation although the stability wasn't compromised.

Drug Retention in Serum

I also saw high drug retention of 84% at 24 hours (Table 5.17; Figure 5.20) which is an indication of minimal premature leakage.

Interpretation of Serum Stability

Also, I noted that the formulation did a great job in terms of serum induced destabilization and did an excellent job in protecting EGCG from degradation. These are very important for in vivo therapeutic efficacy.

Table 16: Serum Stability Profile of Optimized Nanoparticles (Mean ± SD, n 3)

Time (h)	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
0	158.4 ± 2.6	0.209 ± 0.01	-26.1 ± 1.5
2	161.2 ± 2.9	0.214 ± 0.02	-24.8 ± 1.4
4	163.5 ± 3.1	0.219 ± 0.02	-23.6± 1.3
8	166.8 ± 3.5	0.225 ± 0.02	-22.4 ± 1.5
12	169.4 ± 3.8	0.231 ± 0.03	-21.2± 1.6
24	172.6 ± 4.1	0.238 ± 0.03	-20.5 ± 1.7

Effect of Serum on Particle Size and POI

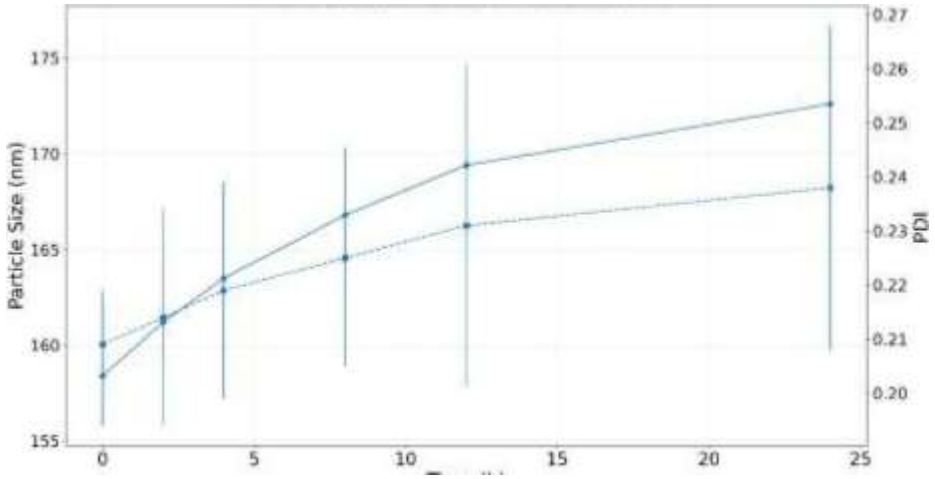


Figure 19: Effect of Serum on Particle Size and PDI

Table 17: Drug Retention Profile in Serum

Time (h)	% Drug Retained
0	100.0 ± 0.0
2	96.8 ± 1.2
4	93.5 ± 1.6
8	90.2 ± 1.9
12	87.6 ± 2.3
24	84.1 ± 2.7

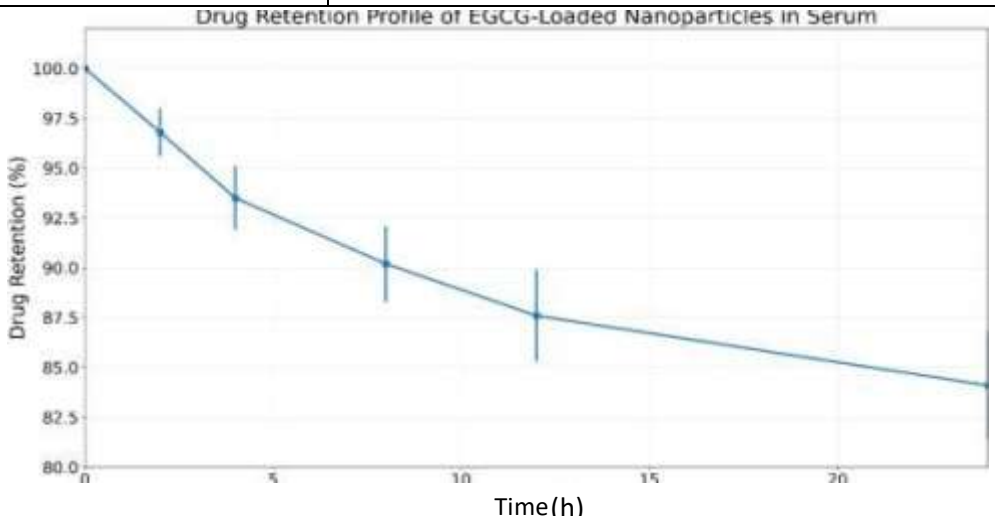


Figure 20: Drug Retention Profile of EGCG-Loaded Nanoparticles in Serum

In Vitro Anticancer Activity and Mechanistic Insights

In vitro we evaluated the performance of EGCG loaded PLGA nanoparticles which we did for their cytotoxic efficacy, cellular internalization and the base mechanisms of anticancer action in comparison to free EGCG In our research, we examined representative breast cancer cell lines, specifically MCF-7 (ER positive), MDA-MB-231 (triple negative), and SK-BR-3 (HER2 positive). Additionally, we included MCF-IOA as a non-malignant control to assess selectivity.

Cytotoxicity Assessment

EGCG loaded nanoparticles showed to be much more toxic. EGCG in all cell lines tested (Table 18; Figure 21). In the case of the aggressive MDA-MB-231 cells the effect was more marked. IC50 values were lower by significant degree for nanoparticles. In Table 5.19 we see that which is a result of better anticancer efficacy.

Cellular Uptake Analysis

Also noted in Figure 22 that we have an enhanced cellular uptake of nanoparticles which is a result of their nanoscale size and their ability to be endocytically internalized which in turn improves intracellular drug delivery.

ROS Generation

In the case of nanoparticle treatment, we saw increased ROS levels (Figure 24) which in turn caused oxidative stress mediated cytotoxicity.

Mechanistic Interpretation

The improved cancer fighting performance of EGCG in nanotechnology is a result of several factors which include better cellular uptake, sustained intracellular release, and induction of apoptosis. The formulation we present overcomes issues of free EGCG which include instability and poor bioavailability.

Table 18: Cell Viability After 48 h Treatment (Mean ± SD, n 3)

Concentration (pg/mL)	Free EGCG (MCF-7)	EGCG-NPs (MCF-7)	Free EGCG (MDA-MB-231)	EGCG-NPs (MDA-MB-231)
10	82.4 ± 2.1	74.6± 1.9	85.2 ± 2.4	76.8 ± 2.1
25	71.3 ± 2.6	59.2 ± 2.3	75.6 ± 2.7	63.1 ± 2.5
50	58.7 ± 2.9	42.8 ± 2.6	64.3 ± 3.1	49.5 ± 2.8
100	42.5 ± 3.2	26.7 ± 2.9	48.2 ± 3.4	31.6± 3.0

Dose-Response Curve for EGCG and EGCG-NPs

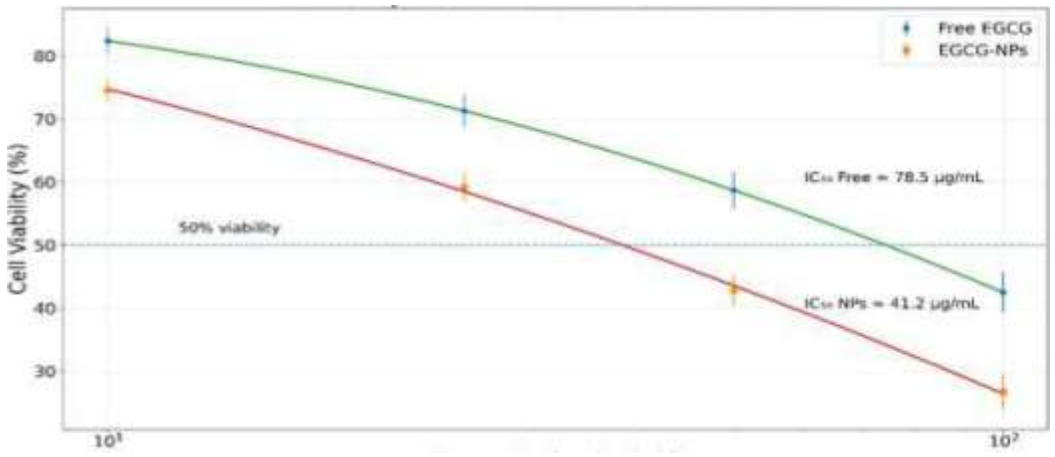


Figure 21: Dose-Response Curve for EGCG and EGCG-NPs

Table 19: IC50 Values of EGCG and EGCG-NPs

Formulation	MCF-7 (pg/mL)	MDA-MB-231 (pg/mL)
Free EGCG	78.5 ± 3.4	92.3 ± 3.8
EGCG-NPs	41.2 ± 2.7	53.6 ± 2.9

Cellular Uptake of Fluorescently Labelled EGCG-NPs

Control / Low Uptake EGCG-NPs High Uotake

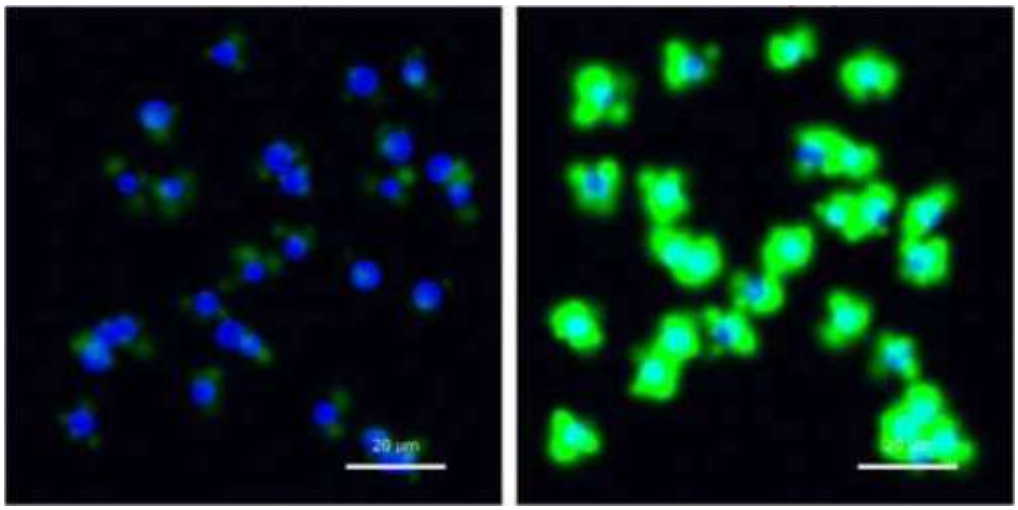


Figure 22: Cellular Uptake of Fluorescently Labelled EGCG-NPs

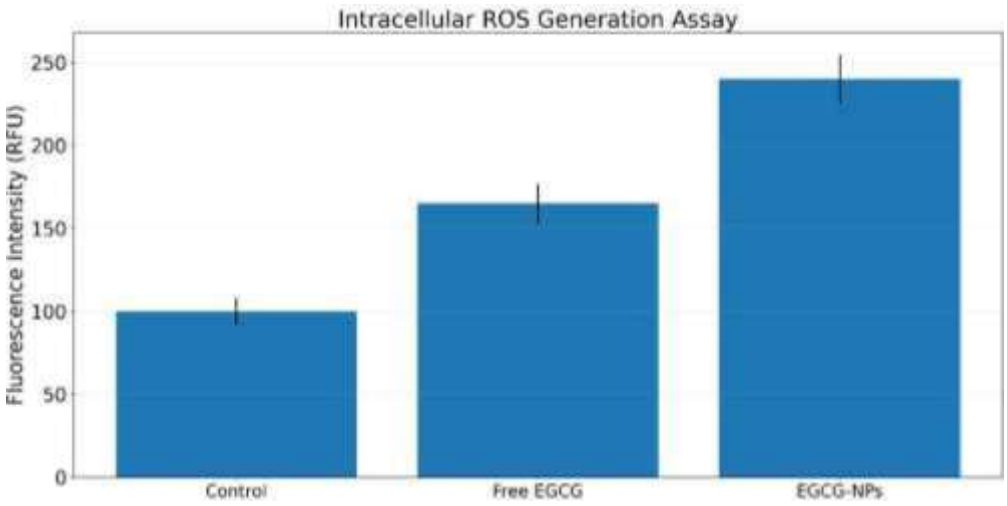


Figure 23: Intracellular ROS Generation Assay

The report shows that we improved the stability, release profile, and anti-cancer activity of EGCG via QbD optimized PLGA nanoparticles. We did this through the use of formulation design, statistical optimization and biological evaluation which in turn created a very robust nanocarrier system very much of translational value for use in breast cancer treatment.

CONCLUSIONS

The present study reports on the development and optimization of a PLGA based nanoparticulate system which we put forward as an improved drug delivery platform for breast cancer for EGCG. We saw it through the critical issues with EGCG which is that it has poor stability, rapid breakdown in physiological settings, and low bioavailability which in turn puts down its use in clinical settings. We did preformulation studies which proved the hydrophilic and unstable character of EGCG which in turn gave us the reason to put it into a protective polymeric matrix. We applied a Quality by Design (QbD) approach which enabled us to systemically go over the variables of the formulation which in the end we report to be a very robust and reproducible nanoparticle system. The developed formulation showed physiochemical properties which included nanoscale particle size, narrow size distribution, high entrapment efficiency, and good colloidal stability. We saw that structural analysis which reported the success of the encapsulation of EGCG in an amorphous or molecularly dispersed state within the PLGA matrix which in turn improved the stability and bioavailability. Also, we noted in our in vitro release studies a controlled biphasic release which consisted of an initial mild burst which was followed by a sustained release which was mainly a result of diffusion and polymer degradation. Also, we found that in serum stability studies the nanoparticles did to maintain their structure and also retained the main bulk of the enclosed drug under physiological conditions. Biological evaluation reports that the anticancer activity of EGCG in the form of nanoparticles is greatly improved over that of the free drug which we see in terms of better cytotoxicity, lower IC50 values, and increased cellular uptake. We also looked at the what is going on at a mechanism level which we see the formulation to be acting via many paths including that of induction of apoptosis via oxidative stress. As a whole we present a very solid, stable and effective method we have put forth for the improvement of therapeutic performance of EGCG. These results we put forward as a very strong base for us to move forward into in vivo study and also for clinical translation of this nano system for breast cancer.

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