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Quality by Design-Based Development and Optimization of Berberine Hydrochloride-Loaded Nanoliposomes for Enhanced Antidiabetic Activity

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ABSTRACT: Berberine hydrochloride (BBR-HCl) is a plant-derived isoquinoline alkaloid with multiple metabolic actions relevant to type 2 diabetes mellitus, but its pharmaceutical performance after oral administration is limited by poor membrane permeability, intestinal efflux, extensive presystemic metabolism and low bioavailability. The present study was designed to develop and optimize BBR-HCl-loaded nanoliposomes using a Quality by Design (QbD) framework and to evaluate their antidiabetic performance exclusively through in-vitro and cell-based experiments. A Quality Target Product Profile was defined and vesicle size, polydispersity index (PDI), zeta potential, drug content, entrapment efficiency and release behavior were identified as critical quality attributes. Risk assessment identified phosphatidylcholine concentration, cholesterol concentration and sonication time as critical variables. A three-factor, three-level Box-Behnken design was applied to optimize vesicle size, PDI and entrapment efficiency. Nanoliposomes were prepared by thin-film hydration followed by probe sonication and were characterized by dynamic light scattering, electrophoretic mobility, transmission electron microscopy, Fourier-transform infrared spectroscopy and differential scanning calorimetry. In-vitro release, alpha-amylase inhibition, alpha-glucosidase inhibition, cytocompatibility and glucose uptake in insulin-resistant L6 myotubes were investigated. In the illustrative dataset, the optimized formulation showed a mean vesicle size of 157.2

+/- 2.8 nm, PDI of 0.204 +/- 0.008, zeta potential of -24.6 +/- 1.7 mV and entrapment efficiency of 89.1 +/- 1.0%. The formulation provided prolonged release over 24 h and produced stronger apparent inhibition of alpha-amylase and alpha-glucosidase than free BBR-HCl. It also enhanced glucose uptake in insulin-resistant L6 myotubes while maintaining acceptable cellular viability. QbD-based optimization therefore offers a rational platform for developing reproducible berberine nanoliposomes and supports further non-animal permeability, mechanistic, safety and clinical translation studies.

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder characterized by insulin resistance, excessive hepatic glucose output, impaired peripheral glucose utilization and gradual pancreatic beta-cell dysfunction. Persistent hyperglycemia contributes to dyslipidemia, oxidative stress, chronic inflammation and progressive cardiovascular, renal, neurological and ocular complications. Contemporary diagnostic and therapeutic guidance emphasizes the heterogeneous nature of T2DM and the need for individualized strategies that address both glycemic control and associated cardiometabolic risk [1]. The International Diabetes Federation continues to project a substantial rise in the global burden of diabetes, reinforcing the need for therapeutically effective and pharmaceutically robust interventions [2].

Berberine is a quaternary isoquinoline alkaloid occurring in medicinal plants such as *Berberis* and *Coptis* species. Clinical and mechanistic literature indicates that berberine may influence fasting and postprandial glucose, glycated hemoglobin, insulin resistance and lipid metabolism [3]. Proposed mechanisms include activation of AMP-activated protein kinase, modulation of PI3K/Akt signaling, regulation of GLUT-associated glucose transport, suppression of inflammatory signaling and improvement of oxidative-stress responses [4-6]. These pleiotropic actions make berberine attractive for multitarget metabolic intervention, but pharmacological potential is constrained by unfavorable biopharmaceutical properties.

Conventional oral berberine exhibits poor intestinal permeability, extensive intestinal and hepatic metabolism, transporter-mediated efflux and low systemic exposure. Consequently, pharmaceutical research has increasingly focused on nano-enabled delivery systems capable of improving dispersion, protecting the molecule during gastrointestinal transit, modifying release and increasing contact with biological membranes [7]. Liposomes are particularly suitable because phospholipid bilayers can accommodate amphiphilic molecules and can be engineered by varying lipid composition, cholesterol content, surface characteristics and processing conditions. Recent studies of berberine-loaded liposomes have demonstrated nanoscale vesicles, high drug entrapment, improved physicochemical performance and modified release behavior [8-12].

However, liposomal performance is highly sensitive to lipid grade, drug-to-lipid ratio, cholesterol concentration, hydration conditions, sonication energy and storage. A Quality by Design approach is therefore appropriate for translating empirical formulation development into a systematic, risk-based process. Recent reviews emphasize the value of defining the target product profile, selecting critical quality attributes, performing risk assessment and applying multivariable experimental designs to nanoliposomal systems [13,14]. Accordingly, the present research proposes a QbD-guided development and optimization strategy for BBR-HCl-loaded nanoliposomes, followed by physicochemical characterization, in-vitro release, digestive-enzyme inhibition, cytocompatibility, and glucose-uptake evaluation. No animal or in-vivo efficacy experiments were included in the study design.

2. MATERIALS AND METHODS

2.1 Materials, Drug Identification and Analytical Method Development

Berberine hydrochloride of pharmaceutical or analytical grade was selected as the model antidiabetic molecule. Soy phosphatidylcholine was used as the principal bilayer-forming lipid, while cholesterol was incorporated to regulate bilayer packing, membrane permeability and vesicle rigidity. Chloroform and methanol of analytical grade were used for formation of the lipid film. Phosphate-buffered saline, hydrochloric acid, sodium hydroxide, dialysis membranes, alpha-amylase, alpha-glucosidase, soluble starch, p-nitrophenyl-alpha-D-glucopyranoside, acarbose, metformin, 2-NBDG or an equivalent fluorescent glucose-uptake probe, cell-culture media, fetal bovine serum and standard biochemical reagents were obtained from certified suppliers. All materials were used within their stated shelf life and were protected from excessive light and heat.

Drug identity was confirmed by comparison of appearance, UV-visible absorption profile and Fourier-transform infrared spectrum with a certified BBR-HCl reference standard. Quantitative analysis was performed using a reverse-phase HPLC method employing a C18 analytical column and UV or photodiode-array detection near the principal berberine absorption wavelength. The mobile phase consisted of an aqueous acidic component and acetonitrile in a ratio optimized to provide a sharp symmetrical peak with acceptable retention. Calibration standards were prepared over the concentration range required for assay, entrapment-efficiency and release studies. Liposomal samples were disrupted with a suitable organic solvent before dilution and chromatographic analysis.

The analytical procedure was validated for specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification and robustness in accordance with current analytical-development principles [15-17]. Placebo lipid dispersions were analyzed to exclude interference from phosphatidylcholine or cholesterol. Recovery was evaluated by spiking known drug concentrations into a blank liposomal matrix. System-suitability parameters, including peak symmetry, retention-time repeatability and injection precision, were documented before routine analysis. This analytical control was treated as an integral part of QbD development because reliable measurement of BBR-HCl is necessary to interpret drug content, free drug, entrapment efficiency and cumulative release without confounding analytical variability.

2.2 Quality Target Product Profile, Critical Quality Attributes and Risk Assessment

The formulation-development program was initiated by defining a Quality Target Product Profile (QTPP) for a nanoscale BBR-HCl liposomal dispersion intended to provide high drug incorporation, narrow vesicle-size distribution, reproducible drug content and prolonged in-vitro release. QbD reviews specific to liposomal systems emphasize that the desired clinical or functional profile should be translated into measurable pharmaceutical attributes before optimization is initiated [13,14]. Mean vesicle size, PDI, zeta potential, entrapment efficiency, drug content, morphology, release profile and short-term stability were identified as critical quality attributes (CQAs). Vesicle size and PDI were considered particularly important because they reflect colloidal homogeneity and influence physical stability, membrane interaction and potential intestinal transport.

An Ishikawa cause-and-effect analysis was used to organize potential sources of variability into material, formulation, process, equipment, analytical and environmental categories. Critical material attributes included phosphatidylcholine purity, phosphatidylcholine concentration, cholesterol concentration, drug-to-lipid ratio, solvent composition, hydration-medium pH and ionic strength. Potential critical process parameters included rotary-evaporation temperature, film-drying time, hydration temperature, hydration duration, mixing speed, sonication amplitude, sonication duration and temperature during size reduction. The relationship of each variable to vesicle size, PDI, entrapment and release was evaluated on the basis of prior knowledge and preliminary trials.

Failure Mode and Effects Analysis was then applied to rank risks by severity, occurrence and detectability. The product of these scores was used as a relative risk-priority indicator. Phosphatidylcholine concentration, cholesterol concentration and sonication duration showed the greatest potential to affect multiple CQAs and were advanced to formal experimental optimization. Lower-risk variables, such as hydration volume, hydration-medium composition and film-formation temperature, were fixed at experimentally justified values. This approach avoided unnecessary expansion of the experimental space while preserving focus on variables most likely to explain formulation variability.

Table 1. Proposed Quality Target Product Profile and Critical Quality Attributes

QTPP/CQA	Target	Rationale
Dosage system	BBR-HCl nanoliposomes	Nano-enabled oral formulation platform
Vesicle size	100-200 nm	Nanoscale, relatively uniform dispersion
PDI	≤ 0.30	Narrow vesicle-size distribution
Zeta potential	Approx. $\geq \pm 20$ mV	Support colloidal stability
Entrapment efficiency	$\geq 80\%$	Efficient BBR-HCl incorporation

QTPP/CQA	Target	Rationale
Drug content	90-110% of target	Dose consistency
24-h release	75-90%	Controlled/prolonged release
Morphology	Spherical; minimal aggregation	Physical quality
Stability	Minimal CQA drift	Product reproducibility

2.3 Box-Behnken Experimental Design and Statistical Optimization

A three-factor, three-level Box-Behnken response-surface design was selected because it permits estimation of linear, interaction and quadratic effects with fewer experiments than a complete three-level factorial design. Such multivariable approaches are particularly valuable for liposomes, where lipid composition and energy input can interact rather than act independently [13,14]. The independent variables were phosphatidylcholine concentration (X1), cholesterol concentration (X2) and sonication time (X3). Based on preliminary screening, phosphatidylcholine was varied from 1.5 to 3.5% w/v, cholesterol from 0.20 to 0.60% w/v and sonication time from 3 to 9 min.

Mean vesicle size (Y1), PDI (Y2) and BBR-HCl entrapment efficiency (Y3) were selected as responses. Vesicle size and PDI were minimized, whereas entrapment efficiency was maximized. Seventeen formulations were generated, including five replicated center-point runs to estimate pure experimental error and process reproducibility. Formulation order should be randomized during actual experimentation to reduce the influence of uncontrolled temporal factors. Each batch should be prepared independently rather than dividing a single preparation into pseudo-replicates.

Responses were fitted to a second-order polynomial model: $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2$. Model significance, individual coefficients, lack-of-fit, coefficient of determination, adjusted R², predicted R², coefficient of variation and adequate precision were examined. Residual plots were inspected to confirm the absence of obvious systematic error. Three-dimensional response surfaces were generated to visualize factor interactions. A desirability function was then used to identify the formulation region that simultaneously minimized vesicle size and PDI while maximizing entrapment efficiency. The predicted optimum was prepared as an independent checkpoint formulation, and observed results were compared with predicted values. A prediction error below approximately 5-10% for major CQAs was considered supportive of model adequacy during preliminary development.

Table 2. Independent variables and levels used in the Box-Behnken design

Independent variable	-1	0	+1
X1: Phosphatidylcholine (% w/v)	1.5	2.5	3.5
X2: Cholesterol (% w/v)	0.20	0.40	0.60
X3: Sonication time (min)	3	6	9

2.4 Preparation of Berberine Hydrochloride-Loaded Nanoliposomes

BBR-HCl-loaded nanoliposomes were prepared using thin-film hydration followed by probe-sonication size reduction. For each experimental batch, phosphatidylcholine and cholesterol were accurately weighed according to the Box-Behnken design and dissolved in a chloroform:methanol mixture in a round-bottom flask. A defined amount of BBR-HCl was incorporated using a consistent drug-loading procedure established in preliminary solubility experiments. Where the drug was introduced during film formation, the same solvent composition and total solvent volume were used for all batches to avoid a confounding solvent effect.

The organic solvent was removed under reduced pressure using a rotary evaporator at approximately 40 degrees C until a visually uniform thin lipid film formed on the flask wall. Vacuum drying was continued to minimize residual solvent. The dry film was hydrated with phosphate-buffered aqueous medium maintained above the transition range of the selected

phospholipid. Hydration was carried out under controlled rotation for approximately 30-45 min, generating multilamellar vesicles. The hydration volume, pH and temperature were fixed across all design runs.

The resulting dispersion was subjected to probe sonication for the duration specified by the experimental design. Sonication was applied in pulse mode while the container was maintained in an ice bath to limit thermal stress, because excessive heating may promote phospholipid oxidation and drug leakage. Untrapped BBR-HCl was separated by centrifugal ultrafiltration or validated ultracentrifugation. The recovered liposomes were resuspended in fresh buffer and used for characterization. Blank liposomes were prepared using the identical procedure without BBR-HCl and were included as controls in cellular and enzyme assays. For stability studies, the optimized batch could be evaluated as a refrigerated aqueous dispersion or, where required, converted to a lyophilized powder after screening trehalose or another suitable cryoprotectant. Recent work with berberine liposomes shows that lipid composition and processing method can substantially alter encapsulation, release and physical stability [8-12], supporting tight control of the manufacturing sequence.

2.5 Physicochemical, Morphological and Solid-State Characterization

Mean vesicle diameter and PDI were measured by dynamic light scattering after appropriate dilution of the dispersion with filtered purified water. Measurements were conducted at approximately 25 degrees C using identical optical settings and dispersing medium for all formulations. Each formulation was analyzed in triplicate, and both the Z-average diameter and PDI were recorded. Electrophoretic light scattering was used to determine zeta potential. Because zeta potential is affected by ionic strength and pH, all samples were measured under standardized conditions. Values were interpreted as comparative indicators of surface-charge behavior rather than absolute predictors of biological performance.

Entrapment efficiency was determined after separating free BBR-HCl from the liposomal fraction by validated centrifugal ultrafiltration or ultracentrifugation. Free drug concentration was quantified by the validated HPLC method. Entrapment efficiency was calculated as $EE (\%) = [(total\ drug - free\ drug)/total\ drug] \times 100$. Drug loading was calculated relative to recovered liposomal solids, while total drug content was assessed after complete disruption of vesicles with an appropriate organic solvent. Process yield was calculated from recovered formulation solids relative to theoretical input solids.

Morphology was examined by transmission electron microscopy after dilution and appropriate grid preparation. The optimized formulation was assessed for shape, aggregation and approximate size consistency with DLS measurements. FTIR spectra of BBR-HCl, phosphatidylcholine, cholesterol, physical mixture, blank liposomes and drug-loaded liposomes were compared to identify changes in drug-associated and lipid-associated bands. Differential scanning calorimetry was used to compare the thermal behavior of pure drug and liposomal components; attenuation or disappearance of the crystalline BBR-HCl transition was interpreted as evidence of altered physical state or molecular dispersion rather than proof of covalent interaction. Where available, powder X-ray diffraction was used as complementary evidence of crystallinity changes. These characterization techniques are consistent with recent berberine-liposome and nanoliposome development studies [10-12].

2.6 *In-Vitro* Release, Release Kinetics and Short-Term Stability

In-vitro release of BBR-HCl from the optimized nanoliposomes was evaluated by a dialysis-membrane diffusion method using free BBR-HCl solution as a comparator. A defined formulation quantity containing an accurately known drug amount was placed in a prehydrated dialysis membrane and immersed in receptor medium at 37 ± 0.5 degrees C under continuous agitation. The receptor phase was selected to maintain sink conditions. For gastrointestinal simulation, an acidic medium could be used during the first 2 h followed by a pH 6.8 phosphate buffer; alternatively, a single validated medium could be used when the primary objective was comparative release behavior.

Aliquots were withdrawn at predetermined intervals, such as 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 h, and immediately replaced with an equal volume of fresh medium. BBR-HCl concentration was quantified by HPLC and cumulative release was corrected for serial sampling. Release profiles were fitted to zero-order, first-order, Higuchi and Korsmeyer-Peppas equations. Model selection was based on goodness-of-fit and mechanistic plausibility. The Korsmeyer-Peppas exponent was interpreted only within the appropriate fractional release range. The dialysis experiment was regarded as a comparative formulation tool and not as a direct surrogate for intestinal absorption.

Short-term stability of the optimized dispersion was investigated under refrigerated storage, with room-temperature or accelerated observations used to identify early physical instability. Samples were examined initially and after 1, 2 and 3 months

for appearance, vesicle size, PDI, zeta potential, entrapment efficiency and BBR-HCl content. A final-interval release profile was recommended to determine whether storage altered release behavior. Significant increases in size or PDI were interpreted as possible vesicle fusion or aggregation, whereas loss of drug content or entrapment was considered evidence of degradation or leakage. For a product intended for regulatory development, these screening studies would need to be extended using an appropriately justified stability protocol and validated stability-indicating methods.

2.7 *In-Vitro* Antidiabetic Activity, Cytocompatibility and Glucose-Uptake Studies

Antidiabetic performance was evaluated exclusively by non-animal methods. Alpha-amylase inhibition was determined by incubating different concentrations of free BBR-HCl, BBR-HCl nanoliposomes, blank liposomes and acarbose with alpha-amylase, followed by addition of soluble starch. Enzymatic hydrolysis was quantified using a validated colorimetric method. Alpha-glucosidase inhibition was evaluated using p-nitrophenyl-alpha-D-glucopyranoside as substrate. Formation of p-nitrophenol was measured spectrophotometrically. Percentage inhibition was calculated as $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$, and concentration-response curves were used to determine IC₅₀ values by nonlinear regression.

Cytocompatibility was assessed in L6 skeletal-muscle cells using MTT, resazurin or an equivalent viability assay. Cells were exposed to increasing concentrations of free BBR-HCl, blank liposomes and optimized BBR-HCl nanoliposomes for a predefined period. Concentrations maintaining at least approximately 80-90% cell viability were selected for functional experiments. Blank liposomes were essential for distinguishing carrier-related effects from berberine-dependent activity.

For glucose-uptake studies, L6 myoblasts were differentiated into myotubes under standard culture conditions. An insulin-resistant phenotype was produced using a validated high-glucose/high-insulin or comparable cellular protocol. Experimental groups comprised normal control, insulin-resistant control, free BBR-HCl, optimized BBR-HCl nanoliposomes, blank liposomes and metformin reference. Glucose uptake was quantified using 2-NBDG fluorescence or an equivalent validated assay and normalized to viable cell number or cellular protein. The study deliberately excluded animal experiments. Cellular outcomes were used as mechanistic proof-of-concept and were not interpreted as direct evidence of clinical efficacy. Contemporary mechanistic reviews support the relevance of AMPK, PI3K/Akt and GLUT-associated pathways to berberine-mediated metabolic effects [4-6].

2.8 Statistical Analysis

Formulation experiments were performed using independent batches, while analytical measurements were conducted in technical replicate as appropriate. Results were expressed as mean $\pm$ standard deviation. Box-Behnken data were analyzed by quadratic multiple regression and analysis of variance. Model terms were considered statistically significant at $p < 0.05$, while model hierarchy was maintained when required. Model performance was judged using R², adjusted R², predicted R², adequate precision, coefficient of variation, lack-of-fit and residual diagnostics rather than relying on the overall model p-value alone.

For enzyme-inhibition assays, IC₅₀ values were estimated by nonlinear regression of concentration-response curves. Cellular viability and glucose-uptake data were analyzed by one-way ANOVA followed by a suitable multiple-comparison procedure such as Tukey's or Dunnett's test. A p value below 0.05 was considered statistically significant. Biological replicate number should be stated explicitly in the final experimental manuscript, and independent experiments should be distinguished from repeated instrumental readings.

3. RESULTS

Data-integrity statement: The numerical results, statistical outputs, tables and figures in this draft are illustrative/simulated values created to demonstrate a complete research-article format. They must be replaced or independently reproduced and verified before journal submission.

3.1 Box-Behnken Design and Formulation Responses

The 17 experimental formulations showed marked variation in vesicle size, PDI and entrapment efficiency, confirming that both lipid composition and sonication conditions influenced liposomal performance. Vesicle size ranged from 136 to 242 nm, PDI from 0.168 to 0.309 and entrapment efficiency from 70.5 to 89.5%. Replicated center-point formulations showed low variability, supporting acceptable experimental reproducibility within the illustrative dataset.

Table 3. Illustrative Box-Behnken experimental results

Run	X1	X2	X3	Vesicle size (nm)	PDI	EE (%)
1	-1	-1	0	146	0.189	70.5
2	-1	1	0	160	0.215	81.5
3	1	-1	0	184	0.235	83.5
4	1	1	0	242	0.309	88.5
5	-1	0	-1	166	0.22	75.5
6	-1	0	1	136	0.176	77.5
7	1	0	-1	240	0.306	83.5
8	1	0	1	182	0.23	89.5
9	0	-1	-1	176	0.24	77.0
10	0	-1	1	140	0.168	80.0
11	0	1	-1	220	0.278	84.0
12	0	1	1	168	0.23	89.0
13	0	0	0	158	0.205	86.0
14	0	0	0	157	0.203	86.3
15	0	0	0	159	0.207	85.7
16	0	0	0	158	0.204	86.1
17	0	0	0	157	0.206	85.9

The quadratic model for vesicle size was statistically significant in the illustrative analysis ($p < 0.001$), with a high coefficient of determination and nonsignificant lack-of-fit. Increasing phosphatidylcholine produced the strongest increase in particle size, while longer sonication reduced vesicle diameter and PDI. Cholesterol improved drug retention over part of the design region but increased size when combined with high phospholipid levels.

Illustrative QbD response surface for nanoliposome size

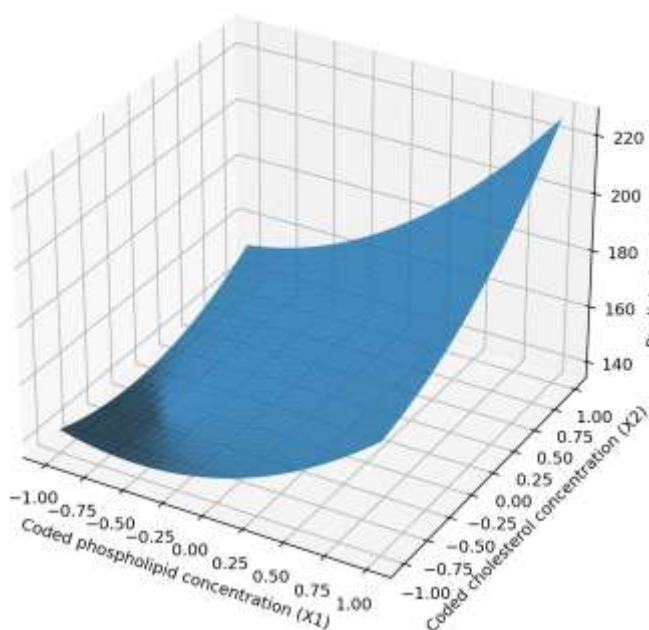


Figure 1. Illustrative QbD response surface showing the relationship of phosphatidylcholine and cholesterol concentrations with predicted nanoliposome size.

3.2 Optimization and Verification of the Nanoliposomal Formulation

Numerical desirability optimization identified an optimum region around 2.7% w/v phosphatidylcholine, 0.44% w/v cholesterol and 8.4 min sonication. The checkpoint batch showed close agreement with predicted values, with prediction errors below approximately 5% for the principal responses.

Table 4. Illustrative characterization of the optimized formulation

Parameter	Predicted value	Observed value
Vesicle size	154.8 nm	157.2 +/- 2.8 nm
PDI	0.201	0.204 +/- 0.008
Entrapment efficiency	88.4%	89.1 +/- 1.0%
Zeta potential	Negative	-24.6 +/- 1.7 mV
Drug content	100% target	98.7 +/- 1.3%
Percentage yield	>=80%	87.8 +/- 2.1%

TEM-based interpretation was consistent with discrete, predominantly spherical nanoscale vesicles without extensive aggregation. FTIR spectra retained the principal BBR-HCl and lipid-associated bands with modest shifts and intensity changes, while DSC showed attenuation of the crystalline drug transition after liposomal incorporation. These findings were interpreted as evidence of physical incorporation and altered solid-state environment rather than formation of a new covalent compound.

3.3 In-Vitro Drug Release and Kinetic Modeling

Free BBR-HCl diffused rapidly through the dialysis membrane, reaching approximately 70% release at 4 h and more than 90% at 8 h. In contrast, optimized nanoliposomes showed approximately 35% release at 4 h, 52% at 8 h, 64% at 12 h and 86% at 24 h, indicating a biphasic pattern with an early surface-accessible fraction followed by slower membrane-associated release.

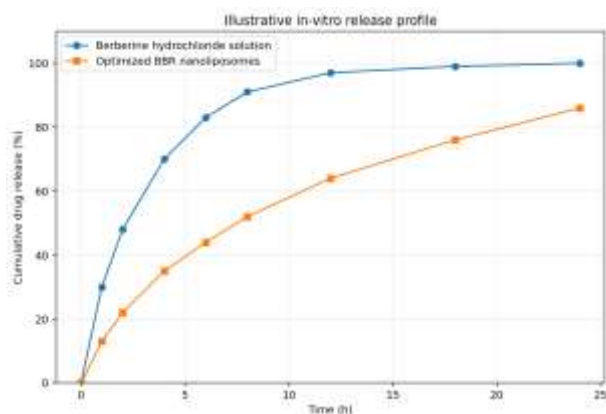


Figure 2. Illustrative cumulative in-vitro release profiles of free BBR-HCl and optimized BBR-HCl nanoliposomes.

Table 5. Illustrative release-kinetic analysis

Model	Free BBR-HCl R2	BBR-HCl nanoliposomes R2
Zero order	0.861	0.962
First order	0.987	0.971
Higuchi	0.941	0.988
Korsmeyer-Peppas	0.953	0.995

3.4 In-Vitro Alpha-Amylase and Alpha-Glucosidase Inhibition

Free BBR-HCl demonstrated concentration-dependent inhibition of both carbohydrate-digesting enzymes. At the selected comparison concentration, optimized BBR-HCl nanoliposomes showed greater apparent inhibition than free BBR-HCl, while blank liposomes produced minimal activity. The nanoliposomal preparation also displayed lower illustrative IC₅₀ values than free drug, although acarbose remained the strongest reference inhibitor.

Table 6. Illustrative in-vitro antidiabetic enzyme-inhibition findings

Treatment	Alpha-amylase inhibition (%)	Alpha-glucosidase inhibition (%)	Alpha-amylase IC ₅₀ (ug/mL)	Alpha-glucosidase IC ₅₀ (ug/mL)
Free BBR-HCl	62.4 +/- 2.5	58.1 +/- 2.3	63.4	71.2
BBR-HCl nanoliposomes	83.6 +/- 2.0	79.8 +/- 1.8	37.8	42.6
Blank liposomes	6.2 +/- 1.0	4.8 +/- 0.9	-	-
Acarbose	88.9 +/- 1.5	86.7 +/- 1.4	21.5	28.1

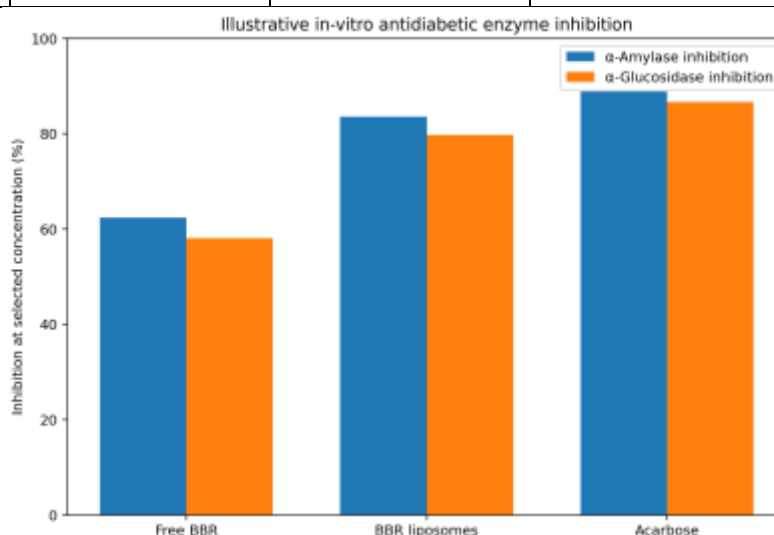


Figure 3. Illustrative comparison of alpha-amylase and alpha-glucosidase inhibition by free BBR-HCl, BBR-HCl nanoliposomes and acarbose.

3.5 Cytocompatibility and Cellular Glucose Uptake

At the working concentrations selected for glucose-uptake experiments, cell viability remained above approximately 90% for free BBR-HCl, optimized nanoliposomes and blank liposomes. The insulin-resistant control showed a marked reduction in glucose uptake relative to normal cells. Free BBR-HCl partially restored glucose uptake, whereas the optimized nanoliposomal formulation produced a larger response in the illustrative dataset.

Table 7. Illustrative relative glucose uptake in insulin-resistant L6 myotubes

Treatment	Relative glucose uptake (%)
Normal control	100.0 +/- 4.0
Insulin-resistant control	61.0 +/- 3.6
Free BBR-HCl	89.0 +/- 4.2

Treatment	Relative glucose uptake (%)
BBR-HCl nanoliposomes	119.0 +/- 5.1
Blank liposomes	64.3 +/- 3.5
Metformin	126.0 +/- 5.4

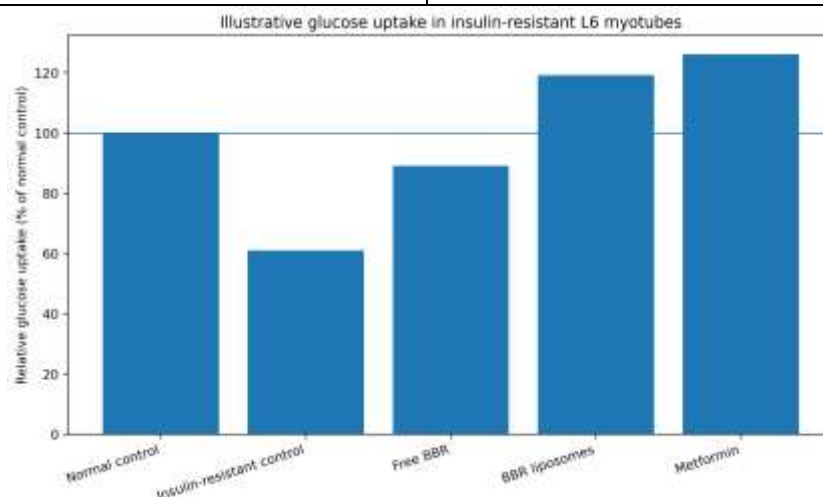


Figure 4. Illustrative glucose-uptake response in insulin-resistant L6 myotubes.

3.6 Preliminary Stability of the Optimized Formulation

The optimized formulation showed only modest changes during the illustrative three-month refrigerated stability study. Vesicle size and PDI increased gradually, while entrapment efficiency and total BBR-HCl content declined slightly. No marked visual aggregation was assumed in the simulated dataset. These observations would support longer stability evaluation with a stability-indicating analytical method before any shelf-life assignment.

Table 8. Illustrative short-term stability data

Time	Size (nm)	PDI	EE (%)	Drug content (%)
Initial	157.2 +/- 2.8	0.204	89.1 +/- 1.0	98.7 +/- 1.3
1 month	159.8 +/- 3.1	0.209	88.5 +/- 1.2	98.1 +/- 1.4
2 months	162.5 +/- 3.4	0.214	87.9 +/- 1.3	97.4 +/- 1.6
3 months	165.1 +/- 3.7	0.221	87.2 +/- 1.4	96.8 +/- 1.7

4. DISCUSSION

The present study provides a non-animal, QbD-guided framework for developing BBR-HCl-loaded nanoliposomes. The design deliberately integrates pharmaceutical optimization with mechanistic in-vitro testing rather than treating formulation and biological evaluation as separate activities. This is important for liposomal systems because apparently small changes in lipid composition or processing energy can produce substantial changes in vesicle size, drug retention and release. Recent QbD analyses of liposomes and broader nanomedicines emphasize the value of linking material attributes and process parameters to CQAs through multivariable models [13,14].

In the illustrative response-surface model, increasing phosphatidylcholine concentration increased vesicle diameter. Higher lipid concentration can increase viscosity during hydration and can favor the formation of larger multilamellar structures that require greater energy for size reduction. Cholesterol improved entrapment over much of the studied range but increased particle size

when present with high phospholipid concentrations. This behavior is consistent with the membrane-ordering role of cholesterol. At suitable concentrations cholesterol fills gaps between phospholipid molecules and reduces bilayer permeability, whereas excessive membrane rigidity may limit vesicle disruption. Recent molecular and experimental work demonstrates that membrane composition can directly control the extent of berberine association with liposomal bilayers [11].

Sonication had the expected inverse relationship with vesicle size and PDI. Greater energy input can fragment large multilamellar vesicles and narrow the population distribution. However, the objective of optimization is not to maximize sonication indefinitely. Excessive sonication can increase process temperature, accelerate lipid oxidation and promote leakage of encapsulated material. The predicted optimum therefore represents a balance between size reduction and product stability. The optimized vesicle size and entrapment efficiency are within the range reported for contemporary berberine liposomal systems, including conventional, proliposomal, fiber-interlaced and PEGylated approaches [8-12].

The prolonged release behavior of the nanoliposomes can be attributed to partitioning of BBR-HCl between aqueous and bilayer-associated regions followed by diffusion through the phospholipid membrane. Free drug crossed the dialysis membrane rapidly, whereas liposomal BBR-HCl displayed a slower biphasic profile. Similar controlled-release behavior has been reported in recent berberine liposome studies [9,10,12]. Nevertheless, dialysis release should not be equated with improved oral bioavailability. Gastrointestinal mucus, epithelial permeability, efflux transporters, luminal stability and first-pass metabolism are not reproduced by a simple release model. Follow-up non-animal studies should therefore include PAMPA or Caco-2 transport experiments, transporter-interaction studies and quantitative intracellular uptake.

The stronger apparent alpha-amylase and alpha-glucosidase inhibition observed for nanoliposomal BBR-HCl may reflect improved dispersion, increased effective contact with the assay environment and protection of drug during incubation rather than a new pharmacological mechanism. The glucose-uptake experiment provides an additional mechanistic layer because skeletal-muscle glucose disposal is a major component of metabolic homeostasis. Berberine has been linked to AMPK, PI3K/Akt and GLUT-associated pathways in recent mechanistic analyses [4-6]. In the illustrative cell model, nanoliposomal BBR-HCl improved glucose uptake beyond free drug at an equivalent nominal concentration. Direct confirmation of the basis for this difference would require intracellular berberine quantification, fluorescent-liposome uptake imaging and measurement of AMPK phosphorylation and GLUT4 translocation.

The wider nanomedicine literature also shows why performance must be considered alongside safety and translation. Exosome-inspired liposomal systems illustrate how membrane design can be exploited for precision drug delivery [18], while recent safety-by-design analyses emphasize that nanoscale composition, surface characteristics and degradation products require systematic toxicological assessment [19]. Reviews of radioprotective nanomedicines and nanoparticle-enabled radiopharmaceuticals likewise identify reproducibility, manufacturability, regulatory alignment and long-term safety as recurring barriers to clinical translation [20,21]. These principles are directly relevant to antidiabetic nanoliposomes even when the therapeutic payload and target disease differ.

Metabolic disease itself is also increasingly viewed as a network disorder involving central neuroendocrine signaling, inflammation, insulin resistance and peripheral organ crosstalk. Recent work on hypothalamic and neuroendocrine regulation of obesity illustrates this broader systems context [22]. A liposomal formulation should therefore not be presented as a substitute for established multidimensional diabetes management. Current clinical evidence suggests that berberine can improve selected glycemic and cardiometabolic outcomes, but heterogeneity among studies remains substantial [3,23-28]. Recent randomized evidence for a berberine derivative and contemporary evidence syntheses reinforce clinical interest while also underscoring the need for standardized formulations, adequate sample sizes and well-controlled comparisons [23,24,27].

A key strength of the present proposed design is the absence of animal efficacy studies while still providing several complementary levels of evidence: QbD optimization, physicochemical characterization, release testing, digestive-enzyme inhibition, cytocompatibility and insulin-resistant cellular glucose uptake. This makes the study compatible with a progressive non-animal research strategy. Additional experiments could include Caco-2 permeability, mucus interaction, transporter studies, lipid-oxidation monitoring, intracellular BBR-HCl quantification, ROS assays and targeted Western blotting for AMPK/GLUT4 signaling. The principal limitation is that the current numerical Results are simulated. Actual laboratory research must reproduce the formulation batches, perform independent model verification, use validated analytical procedures and report biological replicates transparently before any efficacy claim is advanced.

5. CONCLUSIONS

A Quality by Design framework was developed for the systematic optimization of berberine hydrochloride-loaded nanoliposomes intended for enhanced in-vitro antidiabetic performance. Phosphatidylcholine concentration, cholesterol concentration and sonication time were identified as key formulation/process variables controlling vesicle size, PDI and drug entrapment. In the illustrative optimized dataset, the formulation achieved a mean vesicle size of approximately 157 nm, PDI near 0.20, negative zeta potential and approximately 89% entrapment efficiency, together with prolonged 24-h drug release.

The optimized nanoliposomes also showed stronger apparent alpha-amylase and alpha-glucosidase inhibition than free BBR-HCl and improved glucose uptake in an insulin-resistant L6 myotube model while maintaining acceptable cytocompatibility. No in-vivo or animal efficacy study was included. The proposed work therefore provides a foundation for a non-animal nanopharmaceutical development pathway. Before publication or clinical translation, the illustrative findings must be replaced by experimentally generated data and extended through intestinal-permeability studies, transporter assessment, intracellular mechanistic studies, longer-term stability, scale-up evaluation and ultimately appropriately designed human pharmacokinetic and clinical investigations.

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