

ORIGINAL RESEARCH ARTICLE

Statistical Modeling and Process Optimization of Phytosome Production Using the Solvent Evaporation Technique Based on Phospholipid

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ABSTRACT

Fabrication of phytosome formulation using solvent evaporation method statistical modeling, process optimization and scale up evaluation A 3² full factorial was then used for optimal CPP effect study (phospholipid-to-bioactive ratio, sonication time) against desired quality attributes (size, polydispersity index [PDI] and zeta potential of a well-formulated). Statistical analyses indicated that the concentration of phospholipids was the primary determinant of both particle size and colloidal stability, while sonication time modulated particle dispersion uniformity. These differential equations were easily solved, and the resulting $R^2 > 0.99$ predictive power of these mathematical models with little interaction effects suggests that the experimental space was controlled well. Most significantly, a stepwise scale-validation consisted of mass balance and percentage yield at multiple production scales. As the process steps are scaled (from lab to larger batch sizes) there is only a minimal (or no change in encapsulation efficiency and physicochemical stability). The findings of this research demonstrate that in optimized settings, the solvent evaporation process is a mathematically predictable and scalable platform for phospholipid embedded nanostructures to enable delivery of hydrophobic bioactive compounds.

Keywords: Quercetin, Phytosome, Phosphatidylcholine, Factorial Design, Bioavailability, Antioxidant, flavonoi

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1. Introduction

A natural flavonoid called quercetin has anti-inflammatory, anticancer and strong antioxidant effects [1,2]. However, it has poor water solubility and relatively low bioavailability due to its fast metabolism, which limits its clinical applications, although approaches have been taken to resolve these problems [3,4]. Among them, the strategies in which quercetin has been formulated are nanoparticles, liposomes and phytosomes. The use of phytosomes is gaining research interest for targeting a potential role in increasing the bioavailability and solubility hydrophobic bioactive compounds. By simply bonding with phospholipids, the active components of plants form 'phytosome'. These phytosomes provide several advantages with respect to the free compounds including enhanced stability, solubility and pharmacokinetics [5]. The use of phosphatidylcholine, an essential phospholipid, as a vehicle in the phytosome formulation has proven to be successful in encapsulating hydrophobic compounds including quercetin resulting in a stable, biocompatible and bioavailable complex [6,7]. One of the major hurdles in chemical and biochemical engineering is that

hydrophobic bioactive compounds have poor solubility, limited dispersion stability, and sensitivity to environmental conditions. Such constraints have direct repercussions on processing efficiency, product consistency, and scalability in industrial settings [8,9].

Lipid-based drug delivery systems, such as phytosomes (phospholipid complexes), have recently attracted increasing attention as potential platform to improve the physicochemical properties of hydrophobic compounds. Such systems increase the dissemination property and stability by molecular complexation with phospholipids like phosphatidylcholine [10,11].

Although the pharmaceutical performance of phytosomes has already been thoroughly studied, with improvements in solubility and bioavailability [12], relatively little effort has been allocated to their engineering, i.e., identification of crucial production parameters via prior parameter optimization studies, process control within the manufacturer unit operation, and potential scalability study [13,14]. At the level of chemical engineering, determining the effect of operating variables on critical quality attributes is required in order to ensure reproducibility (in relation to product-attributed variability) and support industrial scale-up.

It is one of the most widely used approaches for obtaining lipid-based nanoparticles, as it has relatively simple operating steps and flexible scaling. The process performance is nevertheless at the mercy of strategic variables, such as concentration, mixing conditions and input energy, requiring systematic optimization to ensure product quality [15,16].

In this work, a factorial design methodology is used to determine the influence of phospholipid concentration and sonication duration on fundamental quality attributes such as particle size, polydispersity index and zeta potential. This project is centered on the areas of process optimization, statistical modeling and scale-up evaluation to reproduce an engineering perspective in terms of production of phytosome systems that can be used for hydrophobic compounds.

2. Methodology

2.1. Experimental Design:

The optimization of quercetin-loaded phytosome formulation was carried out using a 3^2 full factorial design with Design-Expert® software. Independent variables to be studied: Two factors of two-level three factors full factorial experimental design, models of concentration of phospholipid (X1) and sonication time (X2) were chosen. The influence of these factors was assessed on three responses, i.e., average particle size (Y_1), polydispersity index (PDI, Y_2) and zeta potential (Y_3). Nine experimental formulations (QP1–QP9) were prepared as per the design of experiments

2.2. Preparation of Quercetin-Loaded Phytosomes

Quercetin and phosphatidylcholine were accurately weighed according to the experimental design and dissolved in 20 mL of absolute ethanol under continuous magnetic stirring (500 rpm) at 40°C for 2 h to ensure complete complex formation. The organic solvent was removed under reduced pressure using a rotary evaporator at 40°C, resulting in the formation of a thin lipid film. The obtained film was hydrated with distilled water and further stirred for 30 min to obtain a uniform phytosomal dispersion. Subsequently, the dispersion was subjected to probe sonication at the predetermined time according to the factorial design in order to reduce particle size and improve homogeneity. All experiments were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation. It was then further evaporated under soft conditions to give a thin dry film. To reduce both dimensionality and inhomogeneities, the suspension was subjected to controlled sonication for a finite duration. The effects of phosphatidylcholine concentration(X1) and sonication time(X2) on CQAs of quercetin phytosomes were studied using a full factorial design 3^2 . Three levels (-1 , 0 and $+1$) of each factor generate nine formulations (QP1 – QP9). This enabled independent and combinatorial

evaluation of the impact of each formulation variable on particle size, polydispersity index (PDI), and zeta potential.

Results: A 3^2 factorial design was used to optimise the phytosome formulation. Phytosome formulations were optimized using Table 1. Phospholipid concentration (X_1 : 100-300 mg) and sonication time (X_2 : 5-15 min) were examined in three levels using coded values for response surface modelling to find the best processing conditions.

Table 1. Different factors with coded values

Value	Factors	
	X1 Conc. of Phospholipid (mg)	X2 Sonication Time (min)
-1	100	5
0	200	10
+1	300	15

Characterization of nine factorial design formulations (**Table 2**) revealed nanoparticle sizes of 195-360 nm, PDI values of 0.23-0.42, and zeta potentials of -17 to -38 mV. Optimal formulations (QP4, QP5, QP7) combining small size (<210 nm), narrow distribution (PDI $\leq$ 0.29), and high stability ($|\zeta| \geq 25$ mV) were achieved at intermediate factor levels, demonstrating the feasibility of quality-by-design optimization for phytosomal systems."

Table 2. Composition of quercetine-loaded phytosomes by using 3^2 factorial designs

Sr. No.	Formulation batches	Mean Particle size (nm)	PDI	Zeta Potential (mV)
1.	QP1	240	0.24	-34
2.	QP2	295	0.25	-23
3.	QP3	360	0.42	-17
4.	QP4	210	0.27	-36
5.	QP5	285	0.23	-25
6.	QP6	332	0.38	-19
7.	QP7	195	0.29	-38
8.	QP8	264	0.26	-26
9.	QP9	315	0.39	-22

The ANOVA results mentioned in Table 3 has a direct connection with phytosome formulation strategy. Also, the extremely influential phospholipid concentration ($p = 0.0002$) confirms that is the most important regulating element to control regulated particle size for each increasing [100mg] coupling equates ([coefficient]) nm in particle size based on the regression equation. Sonication time is also statistically significant ($p = 0.0049$), but far less so in practical terms (as judged by the sum of squares, which is an order of magnitude smaller). Lack of AB interaction indicates that the effects of these factors are additive; an optimal concentration of phospholipid does not require concurrent adjustment of sonication to control particle size. Moreover, the absence of quadratic effects suggests that response surfaces are nearly flat over the observed ranges and linear interpolation as well as scale-up prediction can be performed easily without being worried about local optimum or non-monotonic curvature.

Table 3. ANOVA for quadratic models of mean particle size (Y_1)

Source	Sum of Square	Df	Mean Square	F-value	p-value Prob > F	significant
Model	24365.33	5	4873.07	111.88	0.0013	significant
A-Conc. of Phospholipid	21840.67	1	21840.67	501.44	0.0002	-

B-Sonication time	2440.17	1	2440.17	56.02	0.0049	-
AB	0.000	1	0.000	0.000	1.0000	-
A²	72.00	1	72.00	1.65	0.2888	-
B²	12.50	1	12.50	0.29	0.6293	-
Residual	130.67	3	43.56	-	-	-
Cor Total	24496.00	8	-	-	-	-

The results of ANOVA given in **table 4** showed that the quadratic model is appropriate for mean particle size. The maximum R^2 (0.9947) and adjusted R^2 (0.9858) achieved with minimum number of terms in model indicated excellent overall model fit, while the predicted R^2 (0.9474) shown adequate predictive ability of this derived equation model. The low co-efficient of variance (C.V.) at 2.38% indicated high experimental precision, while the adequate precision value of 29.878 indicates an acceptable ratio since a ratio >4 is desirable, indicating that the model can be used successfully for optimization purposes.

Table 4. Statistical parameters of the quadratic model for mean particle size

Std. Dev.	6.60	R²	0.9947
Mean	277.33	AdjustedR²	0.9858
C. V.%	2.38	PredictedR²	0.9474
PRESS	1288.84	Adeq. Precision	29.878

As has also been found for particle size and aggregation, a phospholipid concentration was the most significant effect ($p = 0.0008$), and PDI had a significant quadratic model ($p = 0.0031$). Sonication time alone was not significant; however, it affected the phospholipid concentration which in return influenced the homogeneity of formulations. Non-linear (A^2) prediction thus provided guidance on the optimal range where a low PDI, nano size value and adequate ZP are identified indicating improved colloidal stability. Shown in **table 5**.

Table 5. ANOVA for Quadratic models of PDI (Y_2)

Source	Sum of Square	Df	Mean Square	F-value	p-value Prob > F	significant
Model	0.042	5	8.400E-003	63.00	0.0031	significant
A-Conc. of Phospholipid	0.025	1	0.025	190.12	0.0008	-
B- Sonication time	1.500E-004	1	1.500E-004	1.12	0.3667	-
AB	1.600E-003	1	1.600E-003	12.00	0.0405	-
A ²	0.014	1	0.014	108.37	0.0019	-
B ²	4.500E-004	1	4.500E-004	3.37	0.1635	-
Residual	4.000E-004	3	1.333E-004	-	-	-
Cor Total	0.042	8	-	-	-	-

The ANOVA for the quadratic PDI model (**Table 6**) verifies perfect model fit.^[19] The R^2 (0.9906) and adjusted R^2 (0.9748) are high, which indicate good fitness of the model, and predicted R^2 (0.8962) is indicative of the good predictive ability of the model. The low coefficient of variation (3.81%) indicates good experimental precision. The obtained precision value (19.092) more than 2 also denotes an optimal signal-to-noise ratio, further confirming the suitability of the model for PDI optimization.

Table 6. Statistical parameters of the quadratic model F-value of PDI for mean particle size

Std. Dev.	0.012	R²	0.9906
Mean	0.30	AdjustedR²	0.9748
C. V.%	3.81	PredictedR²	0.8962
PRESS	4.401E-003	Adeq. Precision	19.092

As shown in **table 7**, the zeta potential was modeled using a quadratic ANOVA model ($F = 254.17$, $p = 0.0004$), and thus can be considered to have been estimated well enough with low residual variance. On a ZS scale the most significant variable was phospholipid concentration ($p < 0.0001$) then sonication time with $p = 0.0039$ for zeta potential respectively. The interaction (AB) and sonication quadratic term (B^2) were not significant but the phospholipid concentration quadratic term (A^2) was ($p = 0.0058$), demonstrating a non-linearity of effect. These results correlate with particle size and PDI data confirming high zeta potential of quercetin phytosomes stability obtained at optimal lipid concentration (10 mg/mL) in combination with sonication time.

Table 7. ANOVA for quadratic models of Zeta potential (Y3)

Source	Sum of Square	Df	Mean Square	F-value	p-value Prob > F	significant
Model	458.92	5	91.78	254.17	0.0004	significant
A-Conc. of Phospholipid	416.67	1	416.67	1153.85	<0.0001	-
B-Sonication time	24.00	1	24.00	66.46	0.0039	-
AB	0.25	1	0.25	0.69	0.4664	-
A²	18.00	1	18.00	49.85	0.0058	-
B²	0.000	1	0.000	0.000	1.0000	-
Residual	1.08	3	0.36	-	-	-
Cor Total	460.00	8	-	-	-	-

ANOVA results for the quadratic zeta potential model are given in **Table 8**. The model was the most significant one, with a very high R^2 (0.9976) and adjusted R^2 (0.9937), which means an excellent fit of the regression model to the data. Low standard deviation (0.60) and coefficient of variation (2.25%) indicate precision, and high adequate precision (42.12) accounts for a strong signal-to-noise ratio. The pseudopredictive R^{2o} (0.9739) value is high, indicating a reasonably good predictive ability.

Table 8. Statistical parameters of the quadratic model F-value of Zeta potential for mean particle size

Std.Dev.	0.60	R²	0.9976
Mean	-26.67	AdjustedR²	0.9937
C. V.%	2.25	PredictedR²	0.9739
PRESS	12.02	Adeq. Precision	42.121

3. Results and Discussion

3.1. Quadratic Equations:

$$\text{Mean Particle size} = +279.67 + 60.33A - 20.17B + 0.000AB - 6.00A^2 + 2.50B^2$$

$$\text{PDI} = +0.24 + 0.065A + 5.000E-003B - 0.020AB + 0.085A^2 + 0.015B^2$$

$$\text{Zeta Potential} = -24.67 + 8.33A - 2.00B - 0.25AB - 3.00A^2 + 0.000B^2$$

Generated contour plot by Design-Expert® software in figure 1) showing the synergistic effect of phospholipid concentration (A) and sonication time (B) on mean particle size of prepared nanoparticles. The gradual color change (toward blue) increases with the value of the particle size, which highlights that under conditions of formulation, the sensitivity of the size domain is from low to high values.

Particle size was mainly depended on phospholipid concentration. A statistically significant increment in mean particle size was noted with the rise of concentration from 100 to 300 mg. This may be due to the improved lipid availability for vesicle formation and particle aggregation, resulting in larger nanoparticles.

The sonication time had a secondary but still appreciable effect on the particle size. At low phospholipid concentration, extended sonication led to reduction of particle size as a result of increased cavitation forces which disrupt particle aggregates and dispersion. At higher phospholipid concentrations, however, the influence of sonication time was weakened indicating that lipid–lipid interactions were stronger than the mechanically induced size reduction by sonication.

The diagonal lines correspond to the product of phospholipid concentration and sonication time. Smallest particle sizes (~220–250 nm) were observed at low phospholipid concentrations and moderate to long sonication times. In comparison, the highest particle sizes (>320 nm) were found for higher phospholipid concentrations regardless of sonication length.

In general, the results validate that exact regulation of phospholipid amount is necessary for obtaining NP with sizes in the size range preferred, and sonication time provides a secondary (supporting) setting to adjust particle size parameters in lipid-based DDS.

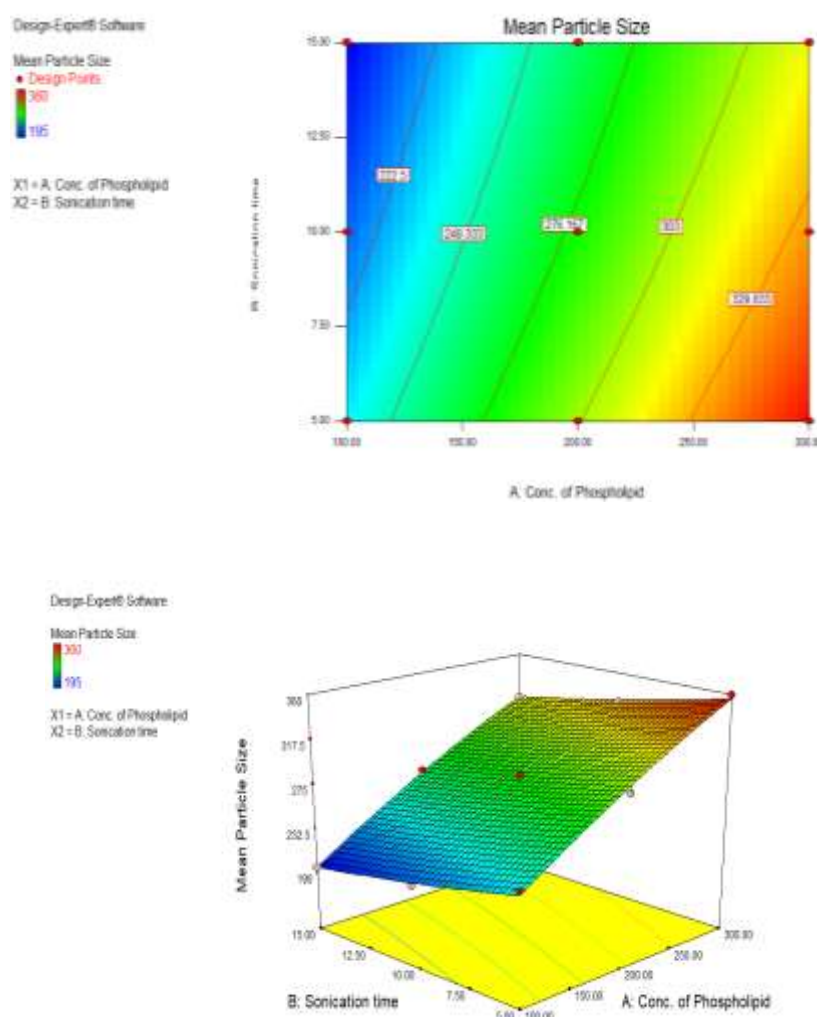


Figure 1. Counter plot & 3D Response Surface for Mean Particle Size

The interaction of phospholipid concentration (Factor A) and sonication time (Factor B) on the PDI of optimized nanoparticles are represented in the contour plot generated by Design-Expert® software shown in figure (2). The colour scaling is given by PDI value from low (blue) to high (red), corresponding to different uniformity of particle size distribution.

The data indicate that the vary concentration of phospholipid has a significant impact on PDI. At low to moderate concentrations of phospholipids (100–200 mg) relatively low PDI (~0.23–0.26) was detected

indicating a good homogeneity of the formulation and narrow size distribution respect to the particle. Conversely, at $> 4 \mu\text{M}$ basal phospholipid a larger PDI was recorded suggesting much broader size distributions due to increased particle agglomeration and heterogeneity.

The mass ratio had a relatively small effect on PDI ($P \leq 0.05$ at phospholipid concentration of 2mg/ml) and the sonication time exerted similar effects, particularly for lower phospholipid concentrations. Increased PDI with longer sonication was caused by an increase in cavitation mechanism for dispersion and disintegration of particle agglomerates. However, at elevated phospholipid levels, sonication offered only limited ability to reduce PDI suggesting that lipid–lipid interaction dominates in reducing the efficiency of dispersion.

1) Contour lines are oval-shaped and are interpreted as an interaction between the concentration of phospholipid and sonication time. Reduced PDI (e.g. > 0.35) at low levels for all phospholipid concentrations and also Higher PDI (> 0.35) obtained at high level for both concentration of phospholipid and intermediate sonication time.

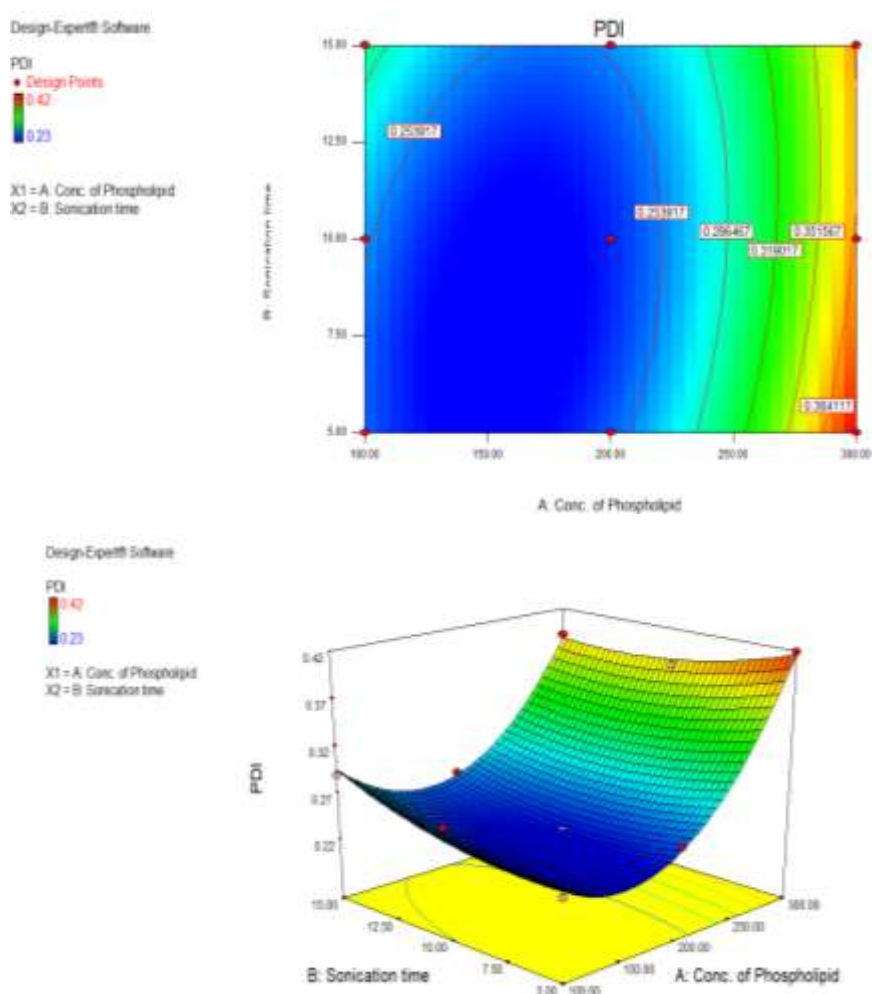


Figure 2. Counter plot & 3D Response Surface for PDI

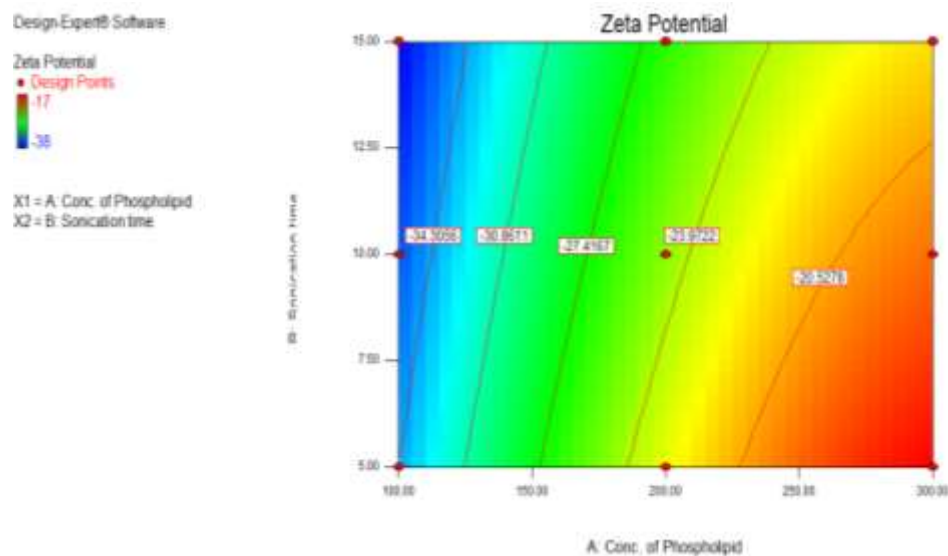
The impact of both phospholipid concentration (Factor A) and sonication time (Factor B) on the mean particle size, PDI and zeta-potential of NPs was conducted using statistical analysis methods offered by Design-Expert® software. The or contour plots that will of how formulation variables interact with their respective effects on nanoparticle performance and stability.

The extent of dependence was high for phospholipid concentration for means of particle sizes. The increase in phospholipid content from 100 to 300 mg resulted in significantly large particle size ($P < 0.05$)

revealing that greater lipid concentrations promote vesicle enlargement and subsequent aggregation. Increasing sonication time was less important: at lower total phospholipids concentrations, smaller particle sizes were obtained with longer sonication times due to improved cavitation and fragmentation processes. At higher, phospholipid concentrations sonication failed to reduce the size of vesicles indicating that instead of a dispersive effect by mechanical agitation lipid / lipid associations predominated. Based on the PDI values this too can be validated. At higher phospholipid amounts, the PDI value (>0.35) increased and then became broad relatively to the larger size of EVs and their nonuniformity, which may result from coagglomeration or partial dispersion of particles. The sonication time had a large influence on the decrease of PDI at lower lipid concentrations, while it only affected slightly the PDI at higher levels.

Measurements of zeta potential showed in figure (3) that the concentration of phospholipid was also a controlling factor in determining surface charge and colloidal stability. Nanoparticles formulated with lower phospholipid concentrations showed more negative zeta potential (-34 to -31 mV), suggesting considerable electrostatic repulsion and better stability. Higher phospholipid concentrations lead to zeta potential less negative (-21 to -17 mV), which could be considered as charge shielding and low surface charge density due to the presence of excessive lipid layers. The zeta potential had limited dependence on sonication time, where the higher negative charge was obtained at moderate durations of sonication.

Overall, integrated analysis suggests that low–moderate phospholipid concentration in combination with the ideal sonication time can produce nanoparticles of smaller base dimension and narrow size distribution to obtain negative zeta potential required for physical stability and improved in vivo drug delivery performance. These findings highlight the need for an improved formulation control via a systematic experimental design, targeting nanoparticles with specific and consistent down-scaled characteristics.



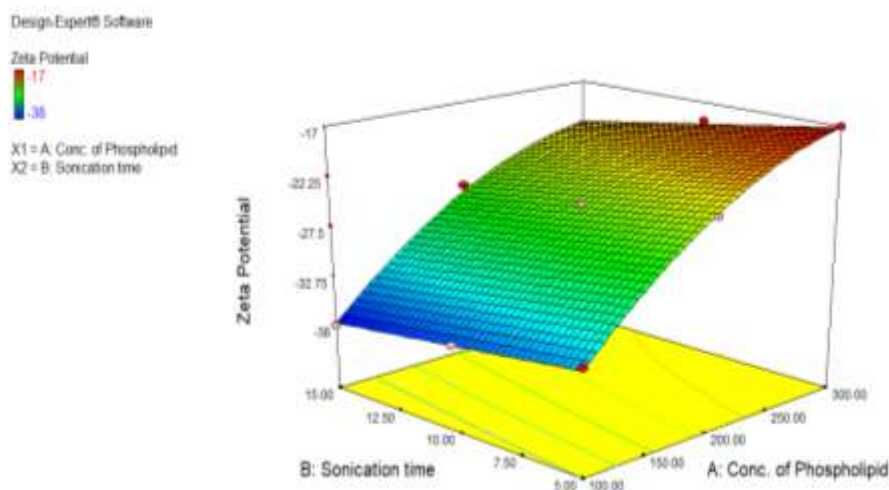
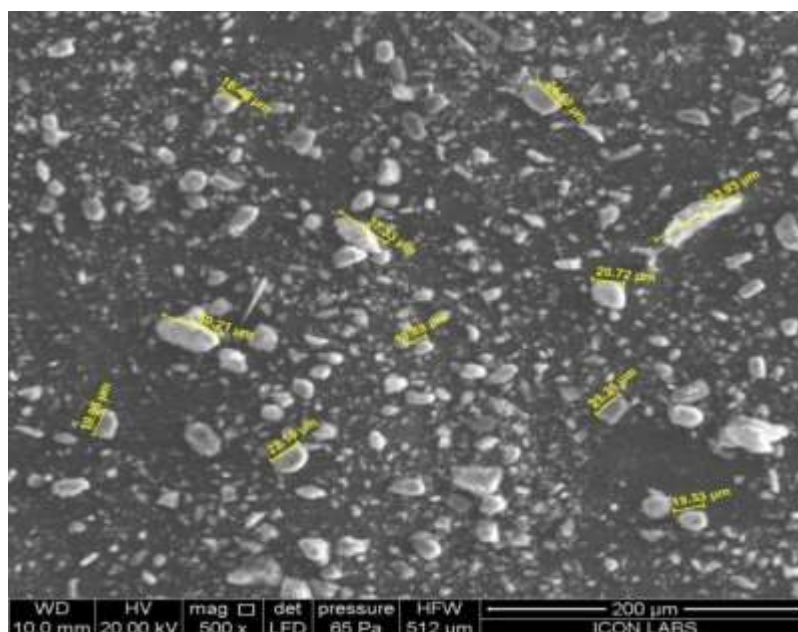


Figure 3. Counter plot & 3D Response Surface for Zeta Potential

The SEM image in fig. (4). of quercetin phytosome formulation reveals the surface morphology and structure of the particles. Smooth spherical-shaped particles with homogeneous structure indicate the entrapment of quercetin in phosphatidylcholine matrix. The approximately particle size (nm) and some agglom were deposited in an effective nano scale of less than 1 μ m, which implies the fact that good formulation stability was produced by these fine dispersions. We can say that the reproducibility of construction is acceptable and more importantly, the phytosome system represents a stable and uniform drug delivery system [17].



demonstrate factorial design as an effective strategy for phytosome optimization and a significant approach to enhancing quercetin bioavailability, thus warranting in vivo investigations.

Abbreviations:

Std.Dev.: Standard Deviation, C.V: Coefficient of variance, R^2 : Coefficient of determination, Adeq.Precision: Adequate precision, Df: Degree of freedom, F-value: Ratio of two variation, *P*-value: Probability value

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