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Pharmaceutical development of phytosomes of Carica papaya leaf extract using the quality by design (QbD) approach

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ABSTRACT

The aim of the research work is to develop phytosomes of *Carica papaya* leaf extract (CPLE) and optimize its formulation with help of Quality by Design (QbD) method. The study involved the preparation of hydroalcoholic extract of fresh leaves. The extract underwent UV-Visible spectrophotometric analysis establishing a standard calibration curve. Phytosomes were formulated using a rotary evaporator method, with varying concentrations of phosphatidylcholine and cholesterol, optimized through a Full Factorial Design and Response Surface Methodology and characterized. The optimization led to the identification of formulation F5, which contained 2% phosphatidylcholine and 0.2% cholesterol, exhibiting the highest entrapment efficiency. Particle sizes ranged from 265 to 322 nm, while zeta potential values were between -21 and -40. Scanning and Transmission Electron Microscopy confirmed the spherical shape and uniform size distribution of the phytosomes. Stability studies showed no significant changes in particle size, zeta potential, or entrapment efficiency over 180 days. The study successfully developed a phytosomal formulation from *Carica papaya* leaf extract, optimized for particle size and entrapment efficiency. The results highlight the potential of the formulated phytosomes as a stable and effective delivery system, providing a promising avenue for future therapeutic applications.

Keywords: *Carica papaya; quality by design; phytosomes; full factorial design; response surface methodology*

1. INTRODUCTION

Phytosomes are a recent advancement in herbal technology, where active herb constituents are enclosed within phospholipids, serving as effective emulsifiers due to their dual solubility. This innovation significantly improves the absorption of herbal extracts in the intestine, addressing variations in botanical bioavailability [1, 2]. Phytosomes, formed by binding flavonoids and terpenoids to phosphatidylcholine, create microspheres or vesicles that shield active constituents from digestive processes. This breakthrough enhances absorption, bioavailability, and tissue delivery, validated through pharmacokinetic and pharmacodynamic studies. Physicochemical properties reveal their dimensions, shape, and solubility characteristics, with biological properties demonstrating superior outcomes over traditional herbal extracts. Advantages include enhanced absorption, simplified formulation, and improved stability. The low bioavailability of hydrophilic phytoconstituents necessitates advanced delivery systems like phytosomes, which encapsulate water-soluble phytoconstituents into lipid vesicles. These formulations, popular for herbs like *Ginkgo biloba* and green tea, address the inefficiency of conventional herbal products, especially in treating liver disorders. By converting hydrophilic compounds into lipophilic ones, phytosomes and liposomes overcome bioavailability limitations, with added benefits from hepatoprotective phosphatidylcholine [1, 3, 4].

Carica papaya, also known as papaya or pawpaw, is a tree belonging to the Caricaceae family. Various parts of the papaya plant have medicinal properties, with reports of anti-malarial and anti-ulcer activities. Papaya seeds are utilized for treating conditions like enlarged liver and spleen, as well as bleeding piles. Indigenous to Central America, Mexico, and parts of South America, papaya is cultivated worldwide, particularly in tropical and subtropical regions. Papaya fruit is purported to

contain linalool, flavonoids, and alkaloids. Fresh papaya leaves are recognized for their therapeutic potential in amoebic dysentery, syphilis, and gonorrhoea [5-9].

Research confirms the presence of phytoconstituents such as carbohydrates, saponin glycosides, amino acids, alkaloids, flavonoids, and phenolics in papaya leaves [11-14]. Studies have investigated papaya leaf extracts for their wound healing, anti-sickling, anti-tumor, and immunomodulatory properties, including their effect on platelet count enhancement in dengue patients [15-20].

Additionally, efforts are made to develop a novel drug delivery system to administer the extract at lower doses effectively. Despite numerous clinical studies demonstrating the platelet enhancement property of papaya leaf juice, challenges persist due to poor bioavailability, large doses, and non-uniformity in herbal dosage forms. Hence, there is a need for modified delivery systems to optimize the performance of herbal formulations. The formulation process, traditionally time-consuming and costly, can be expedited and made more efficient through Quality by Design (QbD) methods, allowing for the optimization of phytosome formulations in a shorter timeframe. In the present study, a full factorial design approach was utilized to optimize phytosome formulations for enhanced efficacy.

2. MATERIAL AND METHODS

Materials

Phosphatidylcholine, phosphate buffer, chloroform and cholesterol were obtained from CDH Fine chemicals, New Delhi. Ethanol was procured from Labogens Fine Chem Industry, Punjab. All the chemicals and reagents utilized in this investigation were of analytical standard.

Experimental

Collection and authentication of Carica papaya leaves

The leaves of *Carica papaya* were procured in August 2020 from the vicinity of Dehra in the Kangra district of Himachal Pradesh. The collected leaves were identified and authenticated by ICAR- National Bureau of Plant Genetic Resources, National Herbarium of cultivated plants, New Delhi (AC-60/2021). Following authentication, the newly obtained leaves underwent thorough washing to eliminate impurities and were subsequently air-dried.

Preparation of Carica papaya leaf extract

The fresh leaves of *Carica papaya* (50 gm) were cleaned, chopped and macerated with hydroalcoholic mixture (100 ml) for 24 hours with occasional shaking. The *Carica papaya* leaf extract (CPLE) were then filtered with muslin cloth and concentrated to dryness. The resultant extracts were stored for further study.

UV-Visible spectrophotometric analysis of CPLE

The CPLE was introduced in phosphate buffer (pH 7.4). To prepare the standard calibration curve for CPLE, dilutions were made from 20-100µg/ml and readings of absorbance were determined at 200-400 nm by utilizing UV spectrophotometer (UV 3000+, Lab India) [21].

Formulation of phytosomes of CPLE

The phytosomes of CPLE was formulated with the help of rotary evaporator method. Different concentrations of phosphatidylcholine and cholesterol were taken as mentioned in Table 1. The mentioned quantities of phosphatidylcholine, cholesterol and CPLE were dissolved in 10 ml of chloroform in RBF and by using rotatory evaporator, the organic solvent was removed and mixture was dehydrated at 37°C with a speed of 120 rpm. The dehydration of solvent leads to formation of phospholipids mixture in form of thin layer. To this thin film, phosphate buffer 7.4 was added. This hydrated mixture was further subjected to sonication for 20 minutes. It results into homogenous mixture of phytosomes which were stored in freezer (2-8^{0}C) for further use [22-27].

Table 1. Detail of Formulations

Formulation code	CPLE (%)	Phosphatidylcholine (%)	Cholesterol (%)
F1	1	1	0.1
F2	1	1	0.2
F3	1	1	0.3
F4	1	2	0.1

F5	1	2	0.2
F6	1	2	0.3
F7	1	3	0.1
F8	1	3	0.2
F9	1	3	0.3

The prepared phytosomes (F1 to F9) were optimized by using Design Expert software. The design for the experimentation utilized was Full factorial design (FFD) with response surface methodology (RSM). According to the preliminary risk assessment, the selected independent variables were amount of phosphatidylcholine (%) and cholesterol (%). The selected dependent variables were zeta potential, particle size and entrapment efficiency of phytosomes.

Characterization of Carica papaya Phytosomes

(1) Particle size and size distribution of prepared phytosomes

The particle size and size distribution of phytosomes were determined by Zetasizer-1000HS (Malvern Instruments, UK). The prepared phytosomes were mixed in phosphate buffer. The samples prepared were examined at the constant temperature of 25 °C in a triplicate manner [27-31].

(2) Zeta potential

Zeta potential of prepared optimized phytosomes was evaluated with the help of Zetasizer-1000HS (Malvern Instruments, UK) [27-31].

(3) Entrapment efficiency (EE)

The EE of phytosomes was evaluated by the ultracentrifugation. The phytosome suspension underwent centrifugation for 30 minutes at 10,000 rpm, and the obtained supernatant was gathered post-centrifugation. These supernatants were further subjected to UV spectrophotometer at 257 nm (UV 3000+, Lab India) for determination of drug entrapped in the phytosomes [27-31].

(4) Scanning Electron Microscopy (SEM)

The surface morphology of optimized phytosomes were understood by SEM (Model no. JSM-6100, JEOL, Ltd. Japan). The prepared phytosomes under observation was photographed using a Scanning electron microscope [27-31].

(5) Transmission electron microscopy (TEM)

TEM (Model no. JEM-F200, JEOL, New Delhi) was used for study of spherical form of vesicle. 10 ml sample of optimized phytosome was stirred and placed on the specimen [27-31].

(6) Stability Studies

The optimized phytosomes were divided into sets of three and subjected to stability studies. For the stability study, the optimized phytosomes were stored at different temperature ranges: In refrigerator (4 ± 2˚C) for 6 months; Room temperature (25 ± 2˚C and 60 ± 5 % RH) for 6 months and In humidity control chamber (40 ± 2˚C/ and 75 ± 5% RH) for 6 months. The physical stability of prepared phytosomes were evaluated at 1st day, 90th day and 180th day and studied for drug entrapment in triplicate manners [27-31].

Statistical analysis

All the values are expressed in Mean ± SEM (n=06). The data was analyzed by two-way ANOVA followed by Tukey's post-hoc comparison test $P<0.05$ compared to Normal group. It is calculated by use of Graph pad Prism software version 5. Also, all the graphs were also procured from graph pad prism software. $P \leq 0.05$ was considered significant.

3. RESULTS & DISCUSSION

UV- visible spectrophotometric analysis of CPLE in phosphate buffer saline

To prepare the standard calibration curve for CPLE, dilutions were made from 20-100µg/ml and readings of absorbance were determined at 200-400 nm by utilizing UV spectrophotometer (UV 3000+, Lab India). It showed linearity at λmax 257 nm. It had the R^2 value of 0.9814.

Development of phytosomes of CPLE

The phytosomes of selected extract i.e. CPLE was formulated with the help of rotary evaporator method. The prepared phytosomes (F1 to F9) were optimized by using Design Expert software.The design for the experimentation utilized was FFD with RSM. According to the preliminary risk assessment, the selected independent variables were amount of phosphatidylcholine (%) and cholesterol (%). The selected dependent variables were zeta potential, particle size and EE of phytosomes. The details of variables are given in Table 2.

Table 2. Details of factors and responses selected for selection of optimized phytosome

	Factor 1	**Factor 2**	**Response 1**	**Response 2**	**Response 3**
Run	**Amount of Cholesterol**	**Amount of Phosphatidylcholine**	**Particle Size**	**Zeta Potential**	**Entrapment Efficiency**
	%	%	nm	mV	%
1	0.1	1	285	-21	63
2	0.1	2	255	-27	69
3	0.1	3	292	-35	70
4	0.2	1	294	-26.5	65
5	0.2	2	265	-30	72.5
6	0.2	3	295	-37	71
7	0.3	1	322	-36	68
8	0.3	2	295	-35	71.5
9	0.3	3	315	-40	71.5

Response 1: Particle Size

The data obtained from the FFD with RSM for particle size is given in Table 3 and Figure 1 and 2.

Table 3. Response 1 (Particle Size)

Source	**Sequential p-value**	**Lack of Fit p-value**	**Adjusted R^2**	**Predicted R^2**	
Linear	0.1910	0.0153	0.1736	-0.3361	
2FI	0.7530	0.0130	0.0698	-1.9666	
Quadratic	< 0.0001	0.4194	0.9777	0.9150	Suggested
Cubic	0.2397	0.5393	0.9857	0.8777	Aliased

Figure 1. Counter plot for particle size

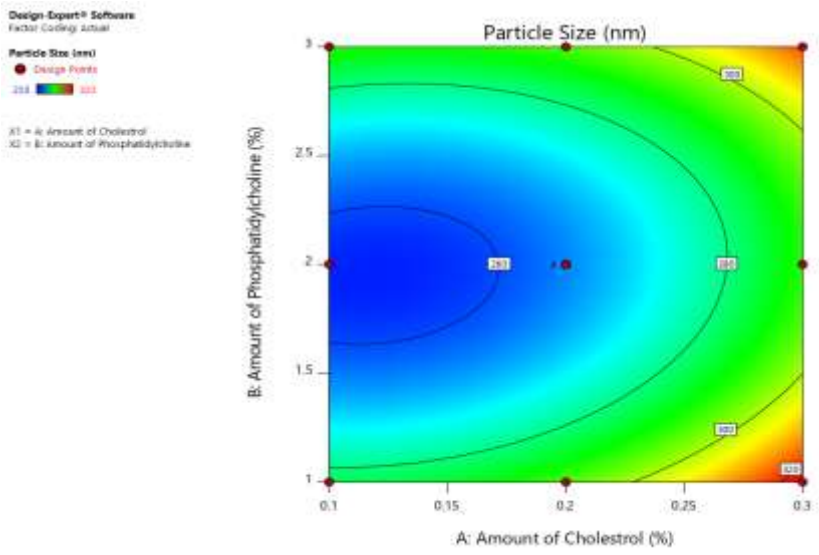
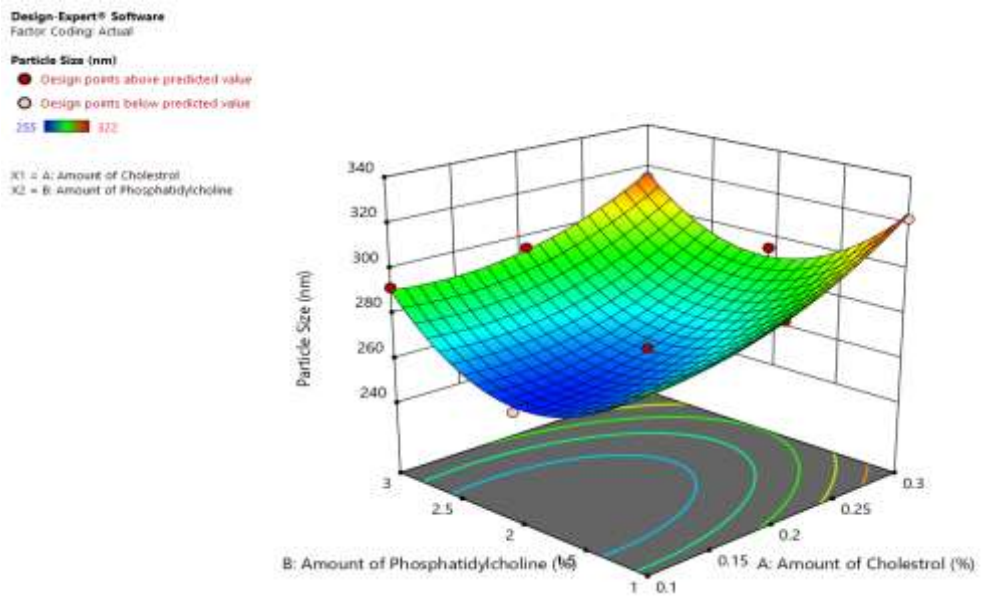


Figure 2. Response surface curve for particle size



Response 2: Zeta Potential

The data obtained from the FFD with RSM for zeta potential is given in Table 4 and Figure 3 and 4.

Table 4. Response 2 (Zeta potential)

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0004	0.0110	0.8185	0.6220	
2FI	0.0216	0.0205	0.9073	0.6758	
Quadratic	0.0042	0.1088	0.9855	0.9279	Suggested
Cubic	0.0209	0.8399	0.9982	0.9972	Aliased

Figure 3. Counter plot for zeta potential

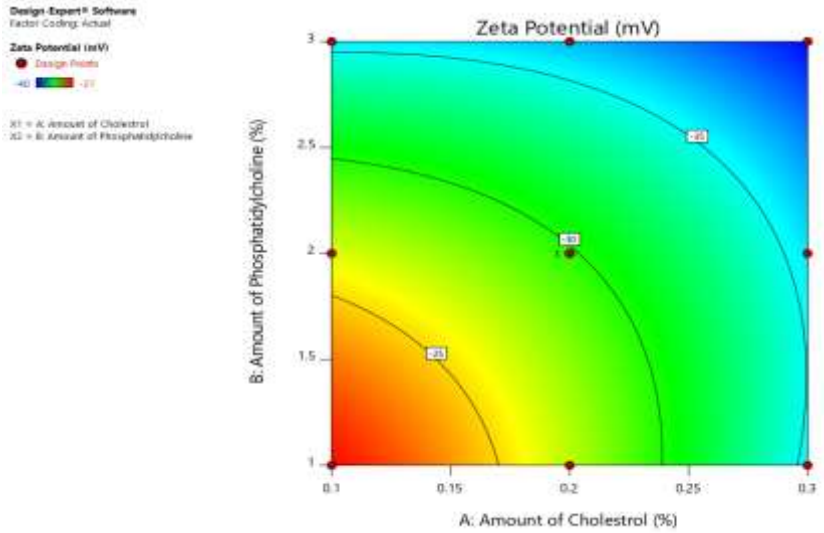
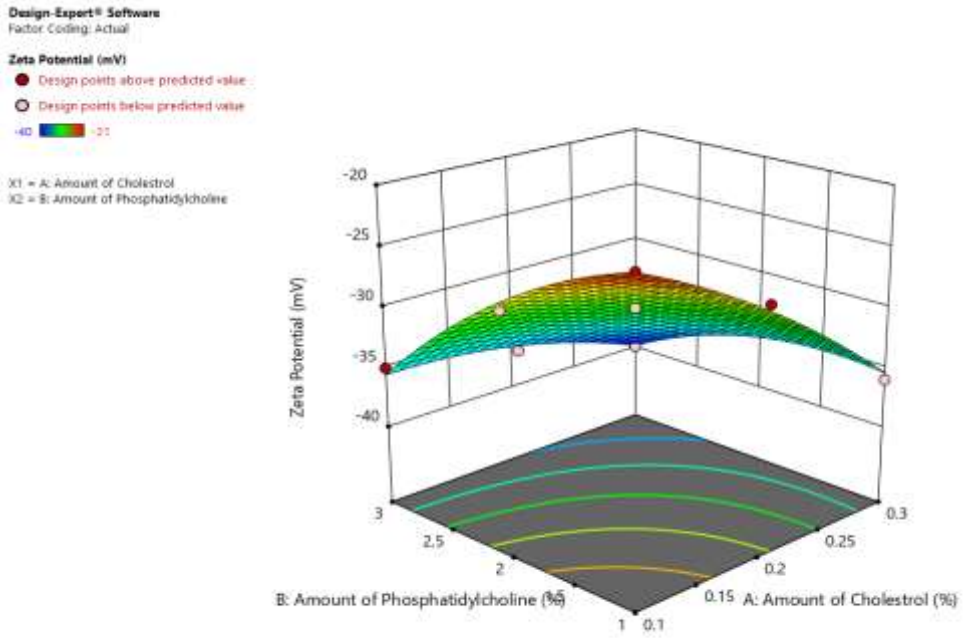


Figure 4. Response surface curve for zeta potential



Response 3: Entrapment Efficiency

The data obtained from the FFD with RSM for entrapment efficiency is given in Table 5 and Figure 5 and 6.

Table 5. Response 3 (Entrapment Efficiency)

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.6418	0.0034	-0.1188	-0.8670	
2FI	0.3565	0.0032	-0.1225	-2.2876	
Quadratic	< 0.0001	0.1739	0.9831	0.9355	Suggested
Cubic	0.4780	0.1031	0.9828	0.4710	Aliased

Figure 5. Counter plot for entrapment efficiency

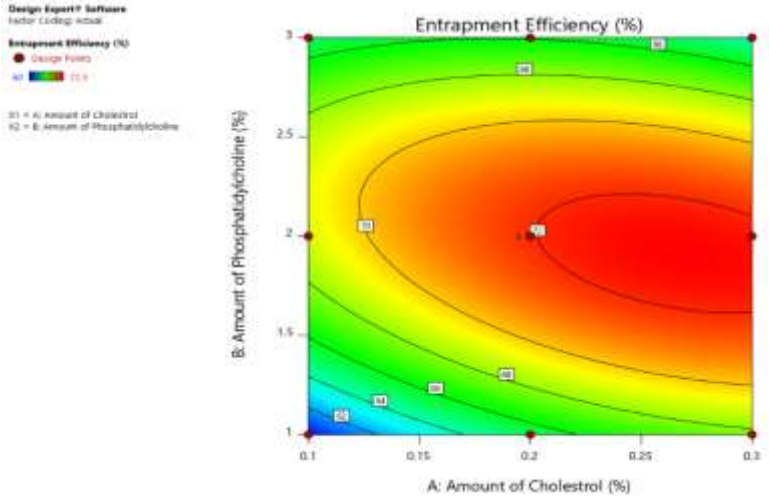
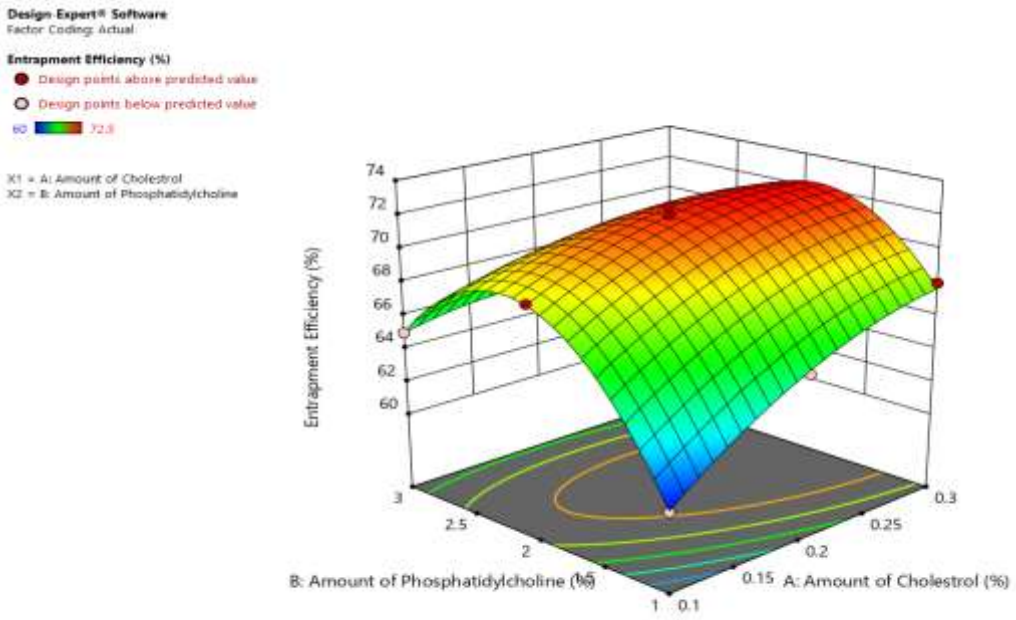
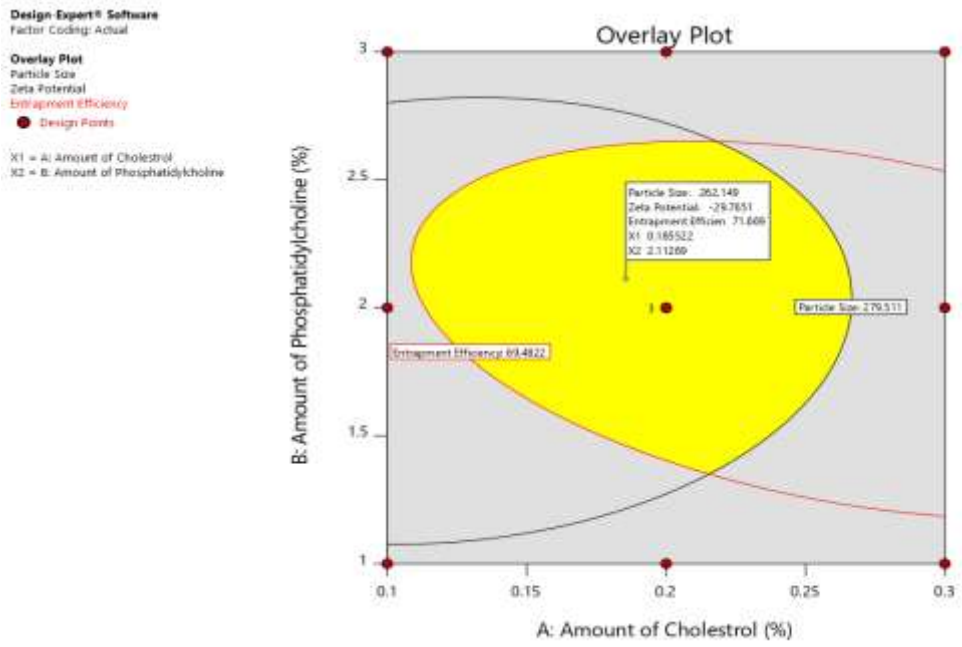


Figure 6. Response surface curve for entrapment efficiency



According to the data provided by Full factorial design with response surface methodology and shown in Figure 7, the optimized phytosome for further investigation is 5th formulation (F5) with amount of phosphatidylcholine and cholesterol 2% and 0.2 % respectively.

Figure 7. Overlay Plot for possible best solution



Characterization of phytosomes of CPLE

Particle size

Particle size for all the 9 prepared phytosomes of CPLE is shown in Table 6. The values of particle size for phytosomes were found to be between 265-322 nm.

Table 6. Particle size of phytosomes of CPLE

Formulations	F1	F2	F3	F4	F5	F6	F7	F8	F9
Average particle size(nm)	285	294	322	294	265	295	322	295	315
PDI	0.275	0.260	0.293	0.288	0.251	0.243	0.321	0.252	0.391

Zeta potential

Zeta potential for all the 9 prepared phytosomes of CPLE is shown in Table 7. The values of zeta potential for phytosomes were found to be between -21 to -40.

Table 7. Zeta potential of phytosomes of CPLE

Formulations	F1	F2	F3	F4	F5	F6	F7	F8	F9
Zeta potential (mV)	-21	-27	-35	-26.5	-30	-37	-36	-35	-40

Entrapment efficiency

Entrapment efficiency for all the 9 prepared phytosomes of CPLE is shown in Table 8. The values of Entrapment efficiency for phytosomes were found to be in the range of 63±1.25% to 72.5±1.19%. The Entrapment efficiency (%) of the formulation F5 was high.

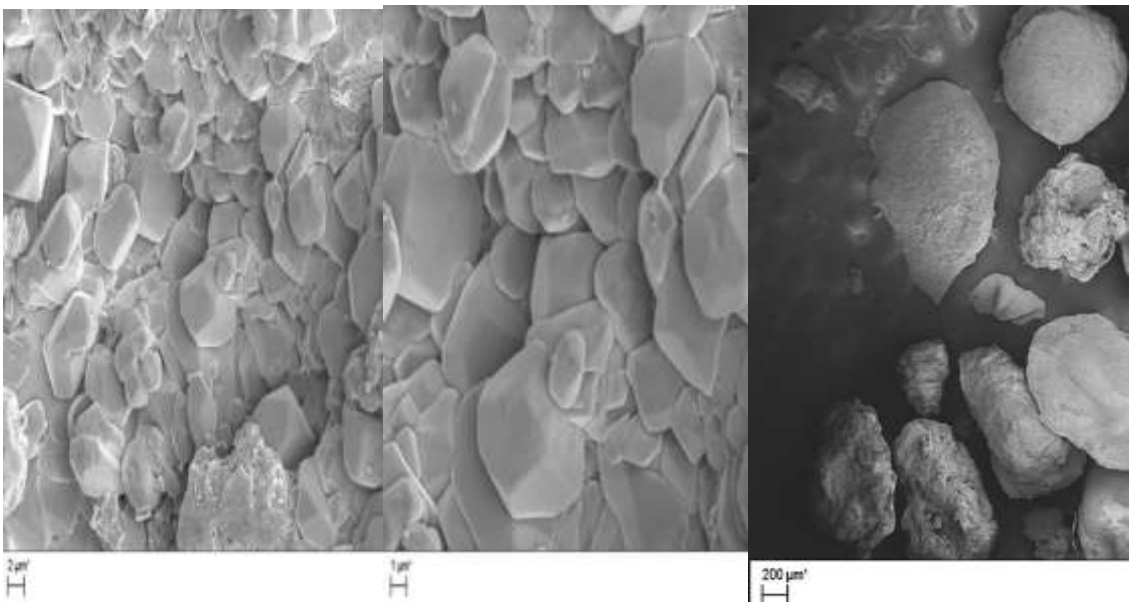
Table 8. Entrapment efficiency of phytosomes of CPLE

Formulations	F1	F2	F3	F4	F5	F6	F7	F8	F9
Entrapment efficiency (%)	63±1.25	69±1.12	70±2.31	65±1.15	72.5±1.19	71±1.27	68±2.13	71.5±1.34	71.5±1.16

Scanning Electron Microscopy

The SEM image of optimized phytosome i.e. formulation F5 is represented in Figure 8.

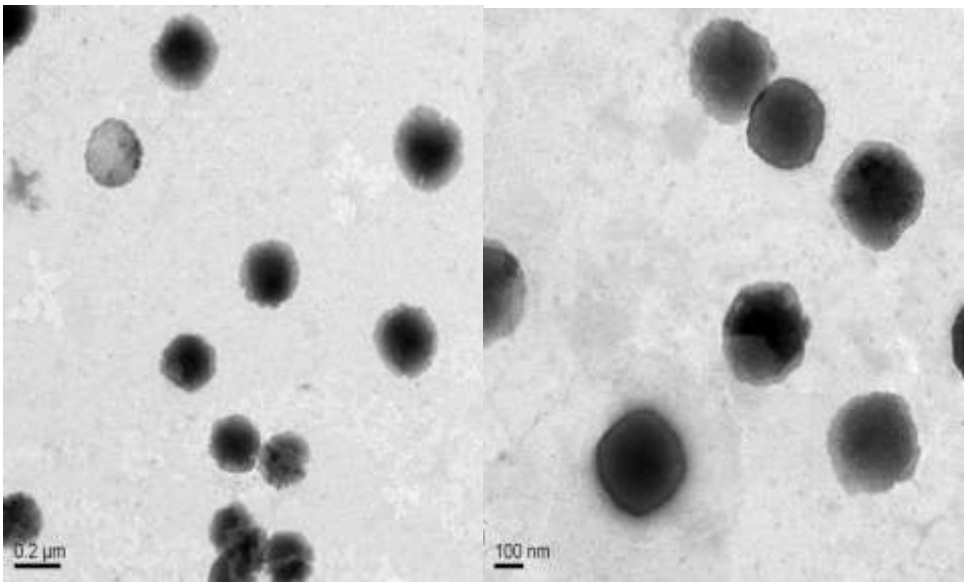
Figure 8. SEM of optimized phytosomes of CPLE



Transmission Electron Microscopy

The TEM image of optimized phytosome i.e. formulation F5 is represented in Figure 9. The phytosomes vesicle showed spherical with core in shape.

Figure 9. TEM of optimized phytosomes of CPLE



Stability study

The optimized phytosomes i.e. formulation F5 were distributed into 3 groups for the stability study and kept at different temperatures. The results obtained from stability study of phytosomes of CPLE are shown in Table 9. The study suggests that the phytosomes were stable in refrigerator, room temperature and in humidity control chamber. There were no significant changes in the readings of particle size, EE and zeta potential after 90 and 180 days.

Table 9. Results of stability study

Parameters	**1st day**	**90th day**	**180th day**
In refrigerator (4 ± 2˚C)			
Particle size (nm)	265 ± 0.2	266 ± 0.1	267 ± 0.4
Zeta potential (mV)	-30 ± 0.1	-32 ± 0.1	-31 ± 0.2
Entrapment efficiency (%)	72.5 ± 0.1	71 ± 0.2	70 ± 1.1
Room temperature (25 ± 2˚C and 60 ± 5 % RH)			
Particle size (nm)	265 ± 0.2	266 ± 0.6	268 ± 0.8
Zeta potential (mV)	-30 ± 0.2	-32 ± 0.2	-33 ± 0.2
Entrapment efficiency (%)	72.5 ± 0.2	70.5 ± 0.3	69.5 ± 1.1
Humidity control chamber (40 ± 2˚C and 75 ± 5% RH)			
Particle size (nm)	265 ± 0.1	267 ± 0.1	269 ± 0.1
Zeta potential (mV)	-30 ± 0.1	-33 ± 0.6	-34 ± 0.5

Entrapment efficiency (%)	72.5 ± 0.2	70 ± 1.2	68.5 ± 1.4

4. CONCLUSION

In summary, a design expert successfully developed and optimized a phytosomal formulation of hydroalcoholic extract from *Carica papaya* leaves. The phytosomes were created using a rotary evaporator method, with varying concentrations of phosphatidylcholine and cholesterol. The formulations (F1 to F9) were optimized using Design Expert software, applying a Full Factorial Design (FFD) and Response Surface Methodology (RSM). The independent variables chosen for the preliminary risk assessment were the amounts of phosphatidylcholine and cholesterol, while the dependent variables included zeta potential, particle size, and entrapment efficiency of the phytosomes.

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) confirmed the spherical shape and uniform size distribution of the phytosomes. Particle size, entrapment efficiency, and zeta potential readings showed no significant changes after 90 and 180 days. The fifth formulation (F5), containing 2% phosphatidylcholine and 0.2% cholesterol, demonstrated the highest entrapment efficiency. Particle sizes ranged from 265 to 322 nm, with entrapment efficiency between 63±1.25% and 72.5±1.19%. The zeta potential values were found to be between -21 and -40. These results indicate that the polyherbal phytosome preparation offers a convenient and safe alternative for dosage form design and delivery.

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Conflict of interest- This study reports no conflict of interest.

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