

Development and characterization of a nano-drug delivery system containing vasaka phospholipid complex to improve bioavailability using quality by design approach

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Abstract

Background and purpose: Vasicine is a potential bronchodilator and can be used for the effective management of asthma and bronchitis. It has low absorption in the gastrointestinal tract due to its poor solubility thereby low bioavailability. The objective of this research was to develop a novel drug delivery system of vasaka extract to improve its bioavailability by enhancing the solubility and absorption of vasicine.

Experimental approach: Vasaka-loaded phytosomes were developed and optimized by thin-layer hydration technique using systematic quality by design approach. Box-Behnken design (3² factorial design) using Design-Expert software was employed to optimize phytosome wherein phosphatidylcholine concentration (X₁), stirring temperature (X₂), and stirring time (X₃) were selected as independent variables. Yield (%), particle size (nm), and entrapment efficiency (%) were evaluated as responses. The optimized phytosome was characterized by studying the surface morphology such as FE-SEM and TEM analysis, thermal characteristics by thermal gravimetric analysis and spectral and diffraction studies by FTIR and XRD analysis and studying the dissolution behaviour of phytosome by *in vitro* release study.

Findings/Results: The percentage yield, particle size, and entrapment efficiency values of the phytosomes were found in the range of 30.03-97.03%, 231.0-701.4 nm, and 20.02-95.88% w/w, respectively. The optimized phytosome showed the zeta potential of -23.2 mV exhibited good stability and SEM and TEM analysis revealed the spherical shape and smooth particles with the uniform particle size distribution of phytosomes. The comparative *in vitro* drug release study of vasaka extract and phytosome revealed the sustained release characteristics of phytosome which reached 68.80% at 8 h compared to vasaka extract reached a maximum of 45.08% at 4 h.

Conclusion and implication: The results highlighted the importance of optimization of formulation development using quality by design strategy to achieve consistent quality of pharmaceutical products.

Keywords: *Adhatoda vasica*; Box Behnken design; Phosphatidylcholine; Phytosome; Vasicine.

INTRODUCTION

Herbal medicine has been widely used since ancient times. Herbal medicines are gaining customer attention by increasing the use of natural products due to the advancement in modern healthcare (1) and believing that are safe to use and more cost-effective when compared to allopathic drugs, especially in people with chronic ailments (2). Advances in the medical field and modernization of

traditional herbal medicine, it is expected to provide an efficient pathway for new drug discovery in the future (3). According to the World Health Organization, about 80% of the population in developing countries still rely on herbal medicine for their primary healthcare needs (4).

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The increasing demand for the use of herbal medicine as alternative therapies needs special attention for researchers to imply pharmaceutical nanotechnology for safe and efficacious medicines. The pharmaceutical applications of herbal drugs, extracts, and bioactive compounds are limited due to low solubility, permeability, as well as bioavailability. It has become a serious hindrance to the effective use of herbal medicine for various health complications. These drawbacks could be resolved by the development of a novel drug delivery system (NDDS) (5).

NDDS has emerged as the most promising innovation strategy (6) to entrap the active constituents inside the lipid layer. They deliver the drug at a rate directed by the requirements of the body, over a period of treatment and potential target of the active compound of an herbal drug to the site of action in a controlled manner to avoid repeated dosing (7,8). It is not only decreasing the repeated drug administration but also has several advantages for herbal drugs including the enhancement of solubility, bioavailability, and sustained release (9), protection from toxicity, enhancement of therapeutic properties, stability, and protection from physical and chemical degradation. Therefore, NDDS of herbal drugs has a potential future for enhancing the activity and overcoming problems associated with conventional DDS (7,10,11).

Among different NDDS, phytosomes are well known biocompatible nanocarriers that can be used to improve the solubility and permeability of phytopharmaceuticals (12). Phytosomes are specially employed for hydrophilic bioactive compounds with low bioavailability and limited absorption, either due to large molecular sizes or very low solubility that retard their diffusion through lipid biomembranes (13). Phytosomes are self-assembled nanocarriers based on the phospholipid complexation approach, which involves the complexation of hydrogen bonding between the phospholipids and phytocompounds. Hydrogen bonding complexation imparts physical stability, enhancing the absorption of phytoconstituents resulting in enhanced bioavailability and superior stability profile of

phytosome when compared to liposomes (12,14).

Phospholipids are structural and functional building blocks of the cell membrane, which have been classified into natural and synthetic phospholipids (15). It has various properties such as amphiphilicity, wettability, emulsifying, solubilizing, and stabilizing effects. Phosphatidylcholine (Phospholipon® 90H) is hydrogenated phosphatidylcholine from soybean particularly recommended as a suitable nanocarrier due to its excellent properties to achieve better absorption and bioavailability to cross the lipid membrane to enter into the systemic circulation (16,17).

Commonly, pharmaceutical industries use traditional process development and drug manufacturing techniques, which fails in ensuring the quality of pharmaceutical products and their desirable aspects (18). Designing a robust formulation requires consideration of various formulations and their process parameters which are critical to the desired quality of the product. Quality by design (QbD) is a systematic, scientific, and risk-based approach that refers to designing and developing a formulation and manufacturing process to ensure the quality of the final product (19-23). Design of experiment is a statistical technique used to obtain an optimum formulation by studying the effect of variables on the response of the experiments and reduces the chance of failure in the product development (24). The design of experiment consists of various designs such as factorial design, Box-Behnken design, central composite design, fractional factorial design, etc. The Box Behnken design consists of a cube of 3-level design having a central point and the middle points of the edges. It determines the independent variables which affect the product or process development. Box Behnken design analyses all the factors simultaneously at high and low levels and avoids experiments under risky conditions (25).

Adhatoda vasica (Syn. *Justicia adhatoda*) is a popular Ayurvedic medicinal plant that belongs to the family Acanthaceae. These are sub-herbaceous bush found throughout the year in plain and sub-Himalayan regions in India (26,27). *A. vasica* is known as vasaka or vasa in Ayurveda and Malabar Nut in English, is an

official drug in Indian Pharmacopoeia and used for the treatment of chronic fever, malarial fever caused by pitta and kapha, intrinsic hemorrhage, cough and asthma, skin diseases, piles, bronchitis, tuberculosis, and leprosy (27, 28). Vasicine, one of the major bioactive pyrroloquinazoline alkaloid present in *A. vasica*, is used as a potential bronchodilator. It also reported to possess oxytocic, abortifacient, uterine stimulant, antioxidant, anti-inflammatory, hypotensive and bradycardia effects (29-32). Due to its poor solubility, reduced permeability, and intestinal instability, vasicine is poorly absorbed and subsequently possesses low bioavailability which seriously restricts its therapeutic applications (33).

In this study, we have reported the application of QbD approach for the development, optimization and characterization of a phyto-phospholipid complex of phosphatidylcholine (Phospholipon® 90H) containing natural extracts (*A. vasica*-alkaloid rich extracts) using thin-layer hydration technique for enhancing oral bioavailability of vasicine.

MATERIALS AND METHODS

Collection of plant material

The fresh matured leaves of *A. vasica* Nees were collected from Chengalpattu (Dt), Tamil Nadu, India and authenticated by Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC), Tambaram, Chennai, Tamil Nadu. The plant specimen, under voucher No. PARC/2018/3653, was stored in the Pharmacognosy Division of Interdisciplinary Institute of Indian System of Medicine (IIISM), SRM Institute of Science and Technology, Kattankulathur, Chengalpattu district, Tamil Nadu, India for future reference.

Chemicals and reagents

Gifted sample of phosphatidylcholine (Phospholipon® 90H) received from Lipoid, Germany. Vasicine (99%) standard was procured from Sigma Aldrich. Methanol, chloroform, ethyl acetate, and dichloromethane were purchased from Ranchem Private Limited, Mumbai and all the other chemicals of analytical grade were used for the preparation of phytosomes.

Preparation of alkaloid extracts

The shade-dried, homogenized-powdered *A. vasica* leaves (100 g) were subjected to cold maceration with methanol. The concentrated methanolic extract was moistened with 5.0% hydrochloric acid and basified to pH 9-10 with concentrated ammonia solution. The basified mixture was further fractionated using hexane (300 mL for 3 times) and followed by chloroform 3 times (each time 50 mL). The chloroform layer was concentrated using a rotary vacuum evaporator (Buchi, Switzerland) under reduced pressure at 50 °C till complete dryness. The obtained alkaloid fraction was stored in the refrigerator at 4 °C until further use (34).

Quantification of vasicine by RP-HPLC-PDA method

The alkaloid-rich extract was quantified for vasicine content using reversed-phase high-pressure liquid chromatography (RP-HPLC) equipped with photodiode array detector method using Shimadzu LC-20AD HPLC system equipped with CT0-20A controller and the column oven. A Rheodyne 7725 injection valve with 20 µL loop volume, an SPD-M20A photo-diode array detector and a Lab solution version 7.1 software were used for data acquisition and interpretation. Standard solution of vasicine 1 mg/mL concentration was prepared with methanol and further dilutions were made with concentration range 20-120 µg/mL. The sample was prepared by taking 100 mg of alkaloid rich extract in 1 mL of methanol. Chromatographic separation was achieved on the Agilent C₁₈ column (150 mm × 4.6 mm; 3 µm pore size). The solvent mixture of 0.1% v/v trifluoroacetic acid (A) and methanol:acetonitrile (45:40) (B) with a ratio of (70:30) at a flow rate of 0.6 mL/min, the column was maintained at room temperature and the PDA detector was fixed at 280 nm for detection of vasicine.

Preparation of phytosomes using thin layer hydration technique

Vasaka-loaded phytosomes were developed by a thin-layer hydration technique. Briefly, differing quantities of vasaka extract and phosphatidylcholine were dissolved in dichloromethane. The mixture was refluxed

and stirred at a specified temperature and time. The final mixture was subjected to evaporation under vacuum at 34-45 °C using a rotary vacuum evaporator until the formation of a thin layer. The thin film was further hydrated with 90% v/v of ethanol in water for 6 h. The formulation was then reserved in a desiccator overnight. The attained extract-phospholipid complex was then shifted into a vial and stored in a freezer for further characterization (35).

Statistical optimization of vasaka-loaded phytosomes using Box-Behnken design

A three-factor and three-level factorial design was employed to optimize diverse critical characteristics influencing the response variables (36). The concentration of phospholipid (X_1), stirring temperature (X_2), and stirring time (X_3) were selected as critical material attributes (CMAs) at three levels i.e. low, medium, and high. Percentage yield (Y_1), particle size (Y_2) and entrapment efficiency (Y_3) were evaluated as dependent quality attributes (CQAs) (Table 1). Total of 15 batches was prepared including three center points. The obtained data were fitted into Design-Expert software (Stat-Ease Design Expert 11.0.2.0, Stat-Ease, Minneapolis, MN). Response surface analyses were carried out and contour plots and 3D-response surface plots were constructed to establish the understanding of the relationship of variables and their interaction. The effect of different CMAs on CQAs was analyzed and second-order polynomial models were established and adapted into multiple linear regression models. Analysis of variance (ANOVA) was used to corroborate the design (37). Restraints for

quality attributes were set at target levels and desirable formulation design was resolved to apply checkpoint analysis and desirability approach using Design-Expert software. The optimum formulation was designated by a numerical optimization procedure using the desirability function (38).

Percentage yield

The percentage yield of the prepared vasaka phytosome was determined using adopting the following equation:

$$\text{Percentage yield (w/w)} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

where, the practical yield is the total weight of the obtained formulation after completion of the experiment and theoretical yield is the sum of the weight of each ingredient used for making formulation.

Determination of entrapment efficiency

The entrapment efficiency was determined using the ultracentrifugation method. The phytosomes (100 mg) was diluted with 1 mL of methanol and subjected to centrifugation on ultracentrifuge at 15000 rpm for 15 min at room temperature. After centrifugation, the supernatant was parted from residue and the supernatant was evaluated for vasicine entrapment using HPLC analysis at 280 nm as per the method given above. To calculate the concentration of free vasicine, the area was replaced into the linear regression of the calibration curve.

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of vasicine} - \text{amount of free vasicine}}{\text{Total amount of vasicine}} \times 100$$

Table 1. Independent and dependent variables in the Box-Behnken design.

Factors		Levels		
		-1	0	+1
Independent variables	X_1 , Phospholipid concentration (g)	1	2	4
	X_2 , Stirring temperature (°C)	40	50	60
	X_3 , Stirring time (min)	60	120	180
Dependent variables	Y_1 , Percentage yield (% w/w)	> 95%		
	Y_2 , Particle size (nm)	< 300		
	Y_3 , Entrapment efficiency (%)	> 95%		

Particle size and zeta potential

The particle size (z-average) and surface charge of vasaka phytosomes were assessed using photon correlation spectroscopy (Zetasizer, Nano-ZS, Malvern Instruments, UK) by the dynamic light scattering method. The samples were diluted in distilled water and sonicated for 5 min. The analysis was executed thrice and the average hydrodynamic particle size was expressed as the value of z-average size \pm SD.

Field emission scanning electron microscopy

The surface morphology of the extract-phospholipid complex was appraised using field emission scanning electron microscopy (FE-SEM; FEI Quanta FEG 200, Netherlands). The powdered sample was kept on an electron magnifying instrument metal stub and coated with gold in a particle sputter. Digital depiction of the sample was taken by irregular scrutinizing of the stub at divergent magnifications. The diameters of the phytosome were indiscriminately measured by FE-SEM images using Image J software.

Transmission electron microscopy

Morphological characteristics of phytosomes were evaluated using transmission electron microscopy (TEM-100S Microscope; JOEL Ltd., Tokyo, Japan). Samples were diluted with ethanol in a ratio of 1:20 and sonicated for 10 min. A drop of phytosome was fixed onto a carbon glazed grid, left to form a thin film and the phytosome pictures were taken using TEM.

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra were obtained from FTIR spectrophotometer (Alpha-T, Universal sample module, Bruker, Germany) by KBr pellet technique. Dehydrated extracts, phospholipids, and optimized phytosomes were ground together with KBr crystals in an agate mortar. The powders were pressed as a disc at a pressure of 15 tons for 10 min in a hydraulic press. The scanning range was fixed at 400-4000 cm^{-1} with a resolution of 2 cm^{-1} .

Thermogravimetric analysis

Thermal stability of the optimized phytosome was investigated using a

thermogravimetric analyzer (TGA; Q50 TA instruments, Waters Pvt. Ltd., Bangalore, India). The extract, phospholipids and phytosomes were loaded on the thermogravimetric-aluminum crucible and then heated at a rate of 10 $^{\circ}\text{C}/\text{min}$ from 30 to 350 $^{\circ}\text{C}$ beneath nitrogen atmosphere. A blank aluminum pan was used as a reference for TGA analysis.

X-ray diffraction analysis

The powder X-ray diffraction (XRD) pattern of the phytosome was achieved by an X-ray diffractometer (D8 Focus, Bruker, Germany) equipped with a Cu-K α radiation source. The experimentation was directed at room temperature in Bragg-Brentano geometry through 2θ ranging from 5 $^{\circ}$ to 80 $^{\circ}$ employing a scanning rate of 0.02 $^{\circ}/\text{min}$. The crystallinity of the phytosome was assessed using origin software.

Solubility studies

The saturation solubility of phytosomes in distilled water and different media were determined using a standard shake flask method (39). An excessive amount of phytosomes were added to distilled water and different buffers with pH 1.2 (HCl), pH 6.8, and pH 7.5 of phosphate buffers. The contents were sealed in a glass vial and placed in a shaking water bath for 24 h at 37 $^{\circ}\text{C}$ at 100 rpm. The samples were then subjected to centrifugation and the supernatant was injected to HPLC analysis to determine the content of vasicine in phytosomes.

in vitro drug release study

The dissolution test was achieved according to the dissolution test method II (paddle) of the United States of Pharmacopoeia (USP 43). To mimic intestinal fluid, phosphate buffer pH 6.8 was used. The dissolution bowls were filled with 900 mL of dissolution media and positioned in a dissolution apparatus to control the temperature at 37 \pm 0.5 $^{\circ}\text{C}$ and the paddle speed was set at 150 rpm in order to achieve uniform dispersion of vasicine. The extract and phytosome (equivalent to 5 mg) were added to the dissolution media and 5 mL of samples were withdrawn at diverse time points (0, 0.08, 0.16, 0.25, 0.50, 0.75, 1, 2, 4, 6, 8, 10, 12, 24 h) and replenished with fresh dissolution media.

The samples were further filtered through 0.22 μm syringe filter and injected into the HPLC system. A New RP-HPLC method was developed for the *in vitro* release study due to the use of buffer as the injecting solution instead of methanol. The method encompassed of RP Phenomenex C₁₈ column (250 mm \times 4.6 mm; 5 μm) with a mobile phase of 0.1% v/v of formic acid in water and methanol (45:55) and a flow rate was set at 0.6 mL/min. The samples were encountered by a PDA detector at a wavelength of 280 nm.

RESULTS

Quantification of vasicine

The bioactive marker and vasicine were identified and quantified in alkaloid rich extracts of *A. vasica* using the RP-HPLC method with PDA as a detector. Construction of calibration curve from the concentration range from 20-120 $\mu\text{g/mL}$ was linear with $y = 15998x - 34475$ and regression coefficient of 0.9989. Vasicine content in alkaloid-rich extract was found to be 5.26 mg/g of dry extract and the HPLC chromatogram of vasicine standard and alkaloid-rich extract was displayed in Fig. 1.

Preparation of phytosomes by Box-Behnken design

The design of experiment is a risk-based assessment to interpret the feasible interactions between the factors influencing the formulation process and quality control of the final formulation. In this study, the Box-Behnken design was enforced to examine the effect of 3 factors namely, phosphatidylcholine concentration, temperature, and time on the

final formulation of phytosomes. Table 1 demonstrates critical material attributes and dependent quality attributes. In the experimental design process, 15 various vasaka phytosomes were established and appraised responses were specified in Table 2.

Characterization of vasaka phytosomes

Percentage yield, particle size and entrapment efficiency

The optimized method revealed the influence of phosphatidylcholine concentration and temperature on the yield, particle size, and entrapment efficiency of phytosomes. The percentage yield and entrapment efficiency of vasaka phytosome increased with a decrease in the amount of phosphatidylcholine added and an increase in the concentration of phospholipids, the yield decreased. A low concentration of phospholipid indicated the minimum particle size. The yield, particle size, and entrapment efficiency values of phytosomes were found to be in the range of 30.03 to 97.03%, 231.0 to 701.4 nm and 20.02-95.88%, respectively. Particle size (231.0 nm) and zeta potential (-23.7 mV) of the optimized phytosomes were depicted in Fig. 2.

The optimization and validation of Box-Behnken design

The desired attributes of vasaka phytosomes were maximum yield, minimum particle size, and maximum entrapment efficiency. The interaction of the factors X₁, X₂, and X₃ on the yield, particle size, and entrapment efficiency was evaluated. The effect of variables on phytosomes was depicted by 2D plots (Figs. 3 and 4) and 3D plots (Fig. 5).

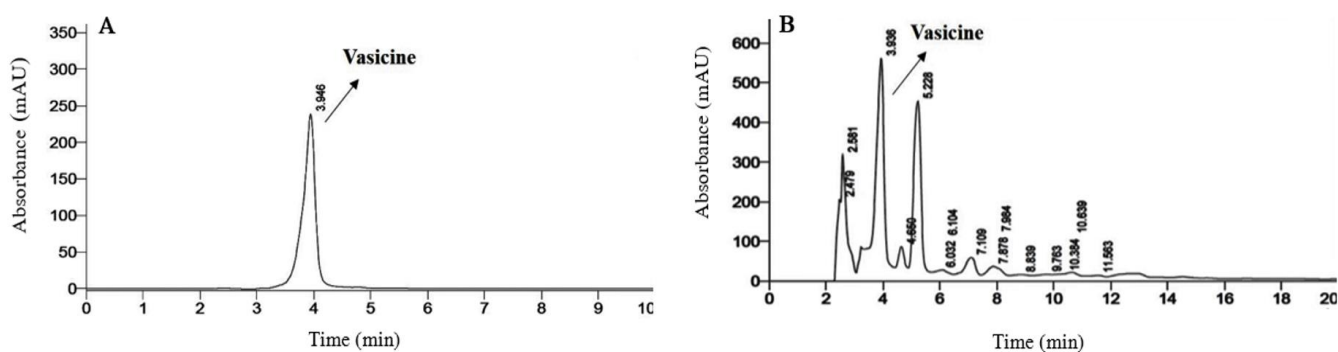


Fig. 1. high-pressure liquid chromatography chromatogram of (A) standard vasicine and (B) alkaloidal extract of *Adhatoda vasica*.

Table 2. Response values of experimental runs in Box-Behnken design

Sample Run	Factor X ₁ Phosphatidylcholine concentration (g)	Factor X ₂ Temperature (°C)	Factor X ₃ Time (min)	Response Y ₁ Yield (%w/w)	Response Y ₂ Particle size (nm)	Response Y ₃ Entrapment efficiency (%w/w)
1	1	40	120	97.03	231.0	95.88
2	2	50	120	74.87	274.3	58.35
3	3	50	60	33.16	614.7	20.02
4	2	50	120	78.16	260.4	39.75
5	2	60	60	71.55	257.5	57.60
6	1	60	120	92.9	275.2	85.79
7	2	60	180	75.25	273.8	40.93
8	1	50	60	95.89	231.4	95.48
9	3	60	120	49.15	353.8	21.24
10	1	50	180	92.56	241.7	88.13
11	3	40	120	31.03	696.3	22.63
12	3	50	180	39.78	288.2	28.37
13	2	40	180	78.98	296.8	52.07
14	2	50	120	79.01	262.9	43.01
15	2	40	60	70.98	701.4	49.66

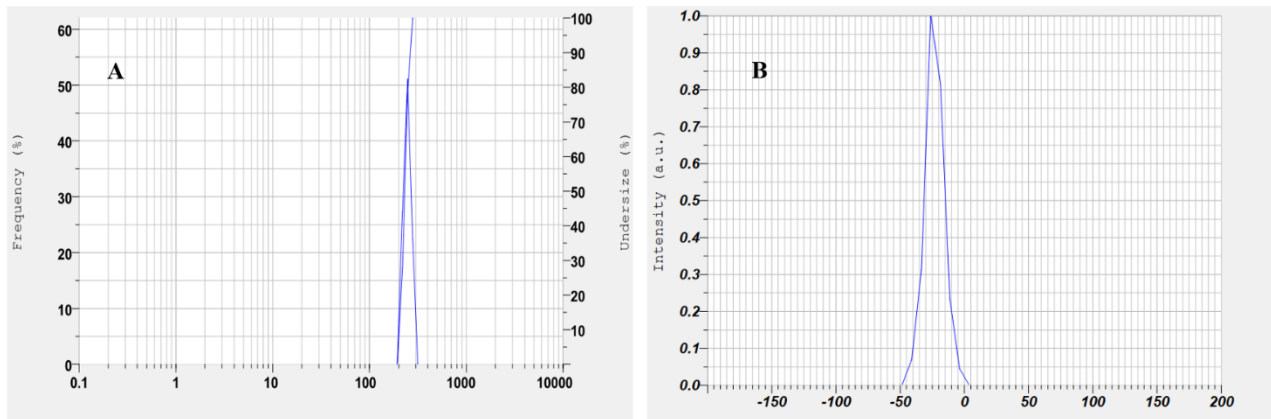


Fig. 2. (A) Particle size and (B) zeta potential of the optimized phytosome.

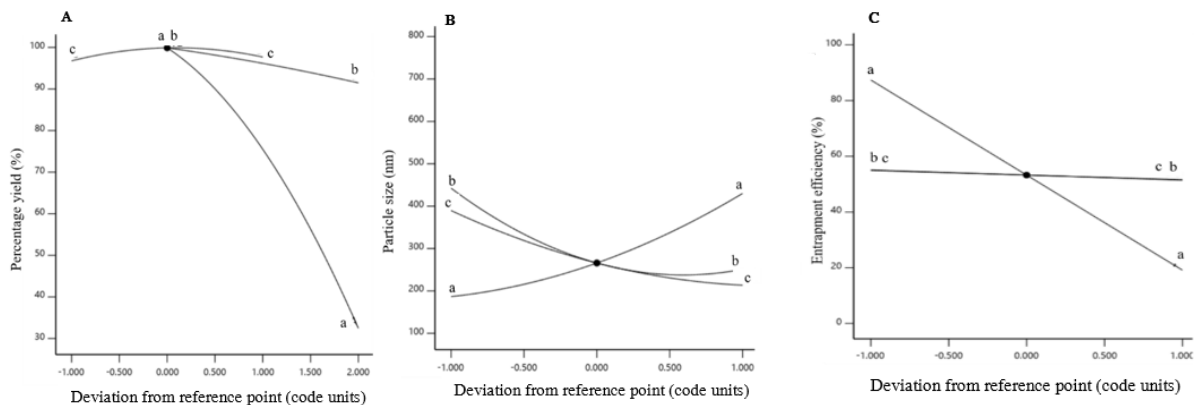


Fig. 3. Perturbation graph for the effect of critical material attributes (a, phospholipid concentration; b, temperature; and c, time on CQAs) on (A) yield, (B) particle size, and (C) entrapment efficiency.

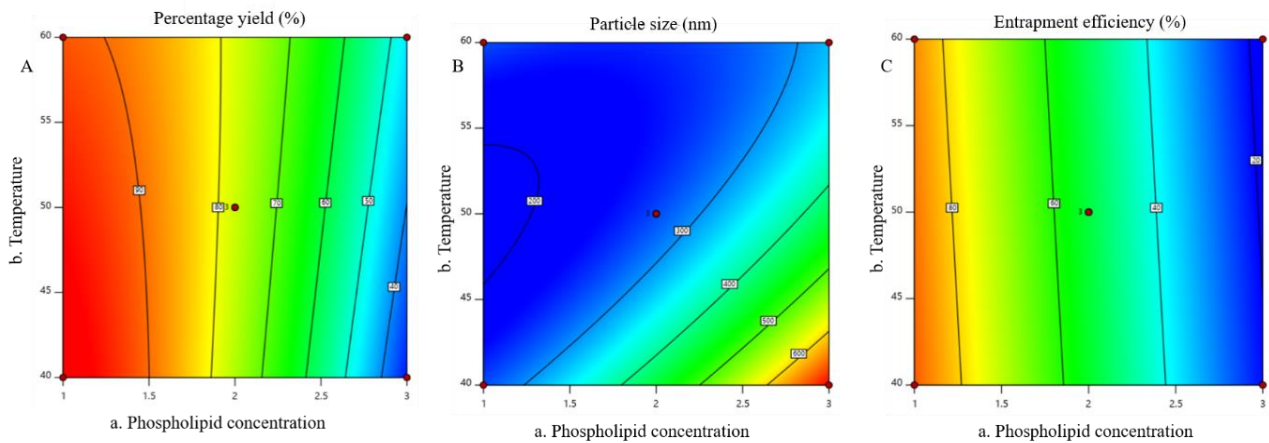


Fig. 4. Contour plots for evaluating the influence of critical material attributes on dependent quality attributes; (A) yield, (B) particle size, and (C) entrapment efficiency

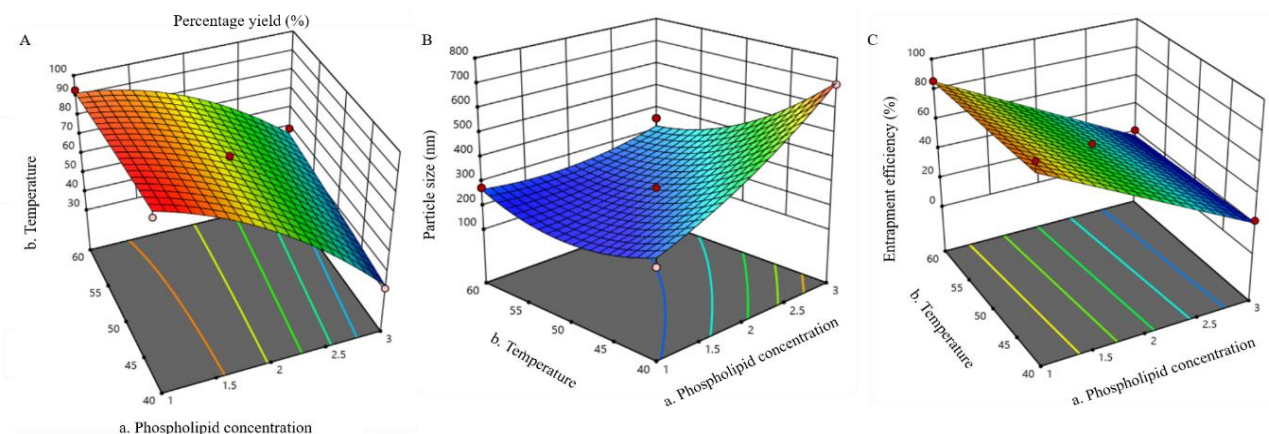


Fig. 5. 3D response surface plots for evaluating the influence of critical material attributes on dependent quality attributes; (A) yield, (B) particle size, and (C) entrapment efficiency.

The phytosome yield was increased with a low phospholipid concentration and low temperature. When the phospholipid concentration was increased, the yield of phytosomes decreased with low temperature. The particle size of the phytosomes was reduced with a low concentration of phospholipid and higher temperature. The entrapment efficiency increased with low phospholipid concentration and low temperature. Stirring time did not show any effect on the yield, and entrapment efficiency but particle size was reduced with longer stirring time. The numerical optimization revealed that the phytosome formulation of extract and phosphatidylcholine ratio of 1:1 at 47.9 and 146 °C for 120 min was achieved the overall desirability of 0.94 as indicated in Fig. 6. The optimized formulation showed the

percentage yield of 95.89%, 231.0 nm of particle size, and 95.48% of entrapment efficiency, which were in consonance with the predicted values.

Surface morphology of vasaka phytosomes

Surface morphology of optimized vasaka phytosome was studied by FE-SEM. Figure 7 illustrates the surface morphology of phytosomes and its average diameter. The micrograph confirmed the oval-shaped uniform vesicles with a smooth surface. The diameters of the phytosomes were calculated using image J software. The average diameter of the phytosome was found to be 226.26 ± 31.97 nm, which affirmed the incorporation of phosphatidylcholine to the extract minimizes the size of phytosomes.

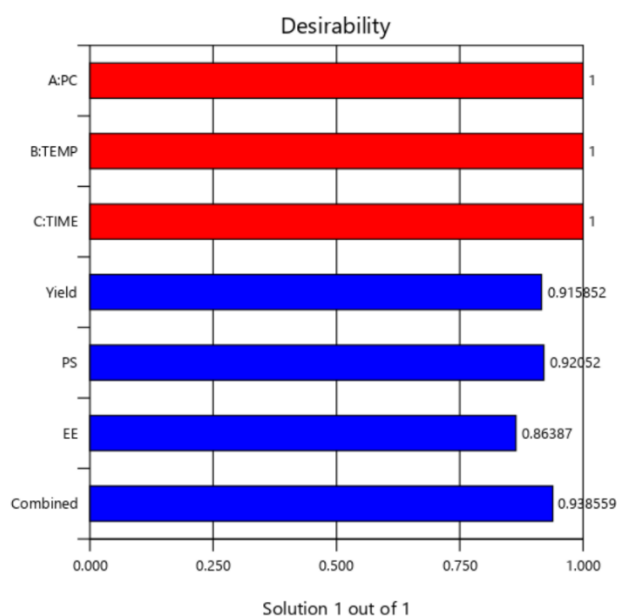


Fig. 6. The desirability plot critical material attributes on dependent quality attributes.

The TEM photograph of the optimized phytosome was exhibited in Fig. 8. The TEM analysis revealed that the phytosomes were sphere-shaped and smooth particles. The internal diameter of the prepared phytosomes was found to be 235.17 nm, which concur with the size attained from SEM images. The particle size distributions of the phytosomes were uniform indicating the non-appearance of aggregation of phospholipids.

FTIR spectroscopy

The FTIR spectra of alkaloidal extract, phospholipon 90H and phytosomes were shown in Fig. 9. The spectra of alkaloid extract revealed characteristic peaks at 3406.69 cm^{-1} (O-H), 2924.89 cm^{-1} (C-H), 2122.28 cm^{-1}

($\text{C}\equiv\text{N}$), and 1614.38 cm^{-1} (C=O). The FTIR spectrum of pure phospholipon 90H conceded the distinctive signals at 3415.49 cm^{-1} (O-H), 2919.00 cm^{-1} , and 2852.43 cm^{-1} , linked to C-H stretching existing in long-chain fatty acids, 1735.84 cm^{-1} and 1649.63 cm^{-1} related to C=O stretching in the fatty acid ester, 1240.88 (P=O stretching), 1092.78 cm^{-1} (P-O-C stretching and 969.52 cm^{-1} [$-\text{N}^+(\text{CH}_2)_3$] (40). The spectra of phytosome showed broad peaks at 3411.84 cm^{-1} (O-H stretching in extract), 1241.11 cm^{-1} (P=O stretching) and 1092.72 cm^{-1} (P-O-C bending). The resemblance of the FTIR spectra exhibited the changes in the definite regions of alkaloid extracts considering the interaction with phospholipids. The difference in the stretching frequency of phenolic OH in alkaloid extract at 3411.84 cm^{-1} of phytosome proposed that feeble intermolecular interaction amidst phospholipids and extracts were attributable to the formation of phytosomes.

Thermogravimetric analysis

Thermal deterioration of pure extract, phospholipon 90H, phytosomes, and its weight loss were shown in Fig. 10. Vasaka extract unveiled 10.55% and 35.36% of weight loss at 183.38 and 313.68 °C, respectively. Phospholipon 90H showed the characteristic weight loss at 193.84 and 284.95 °C with 6.12 and 7.05%. In contrast, the optimized phytosome revealed four characteristic weight losses of 2.87, 8.59, 12.14, and 15.82% at 117.24, 177.09, 253.08, and 303.28 °C, respectively. The derivative weight% of phytosome exhibited the exothermic nature of the phytosomes depicted in Fig. 11.

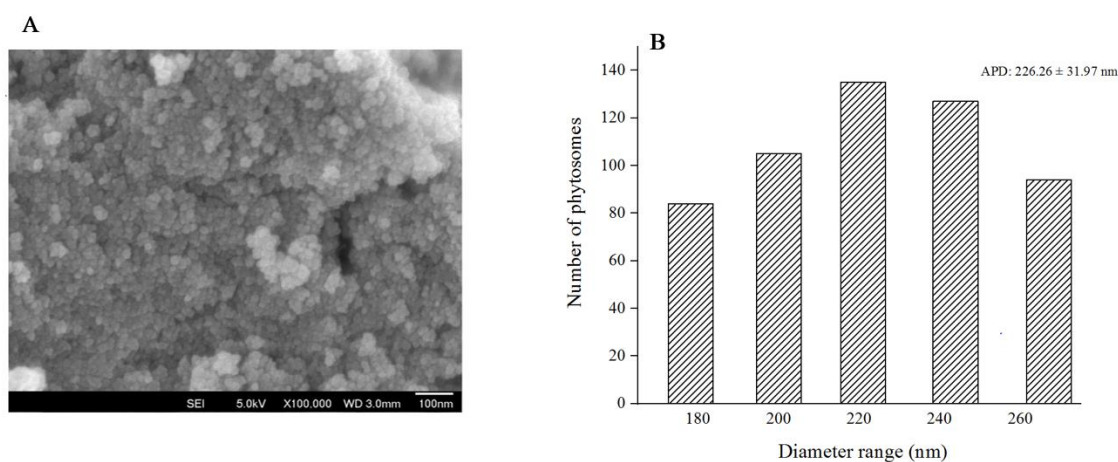


Fig. 7. Surface morphology of vasaka phytosomes by scanning electron microscopy; (A) phytosome and (B) average phytosome diameter.

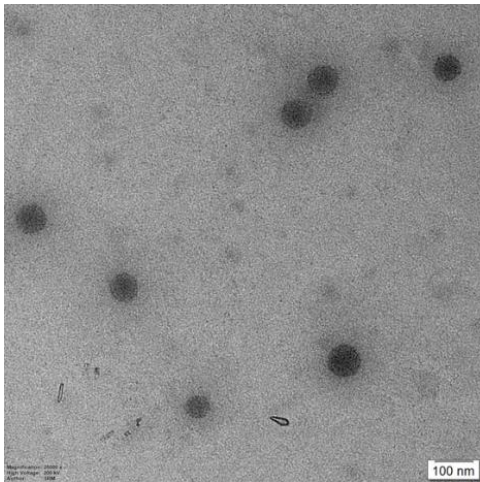


Fig. 8. Transmission electron microscopy images of vasaka phytosomes.

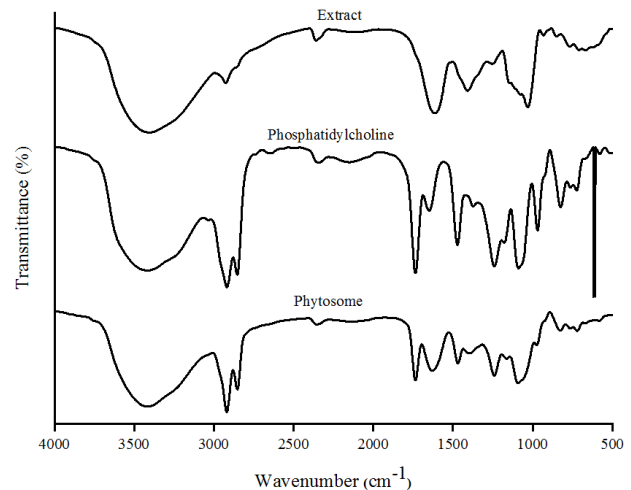


Fig. 9. Fourier transform infrared spectra of alkaloid extract, phosphatidylcholine and phytosome.

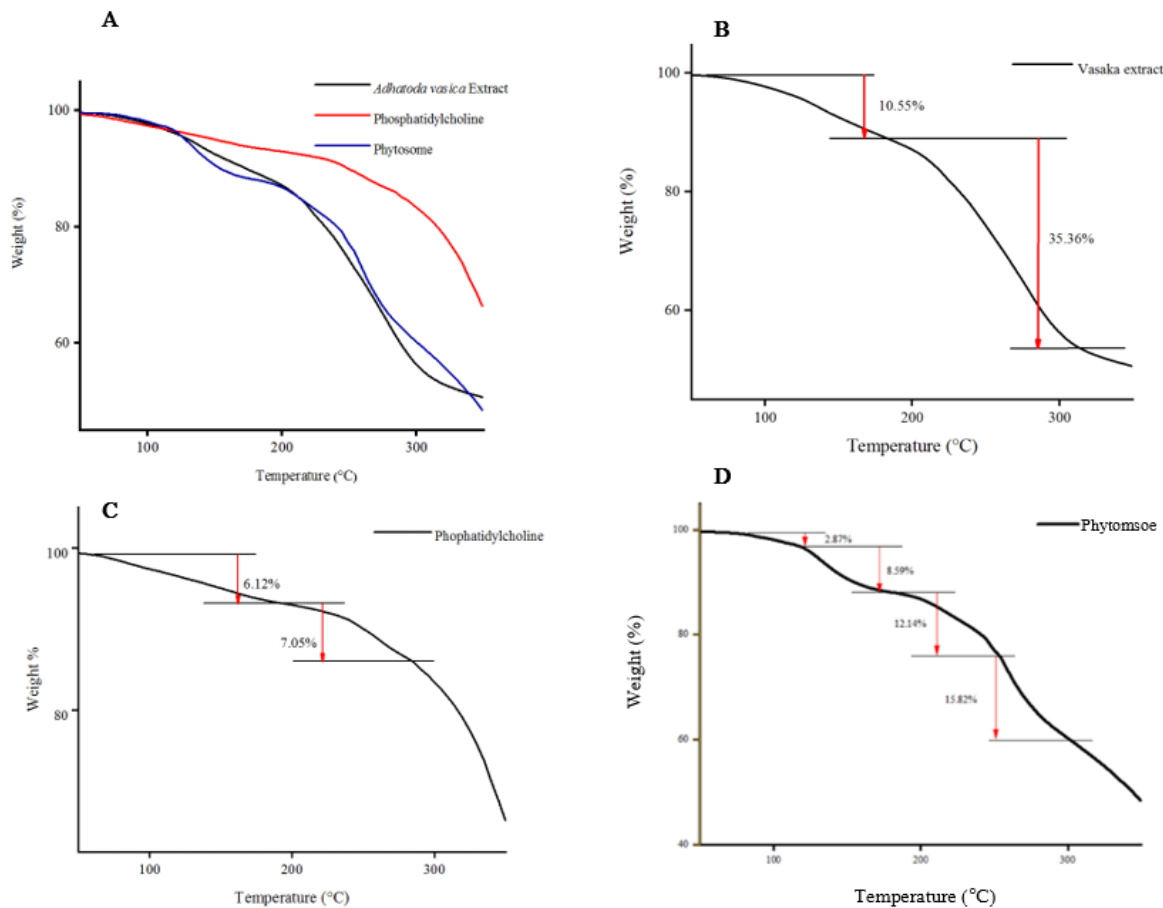


Fig. 10. Thermogram of (A) vasaka extract, phosphatidylcholine and phytosome; (B-D) percent of weight loss of *Adhatoda vasica* extract, phosphatidylcholine, and phytosome, respectively.

Powder X-ray diffractometry

The X-ray diffractogram of optimized phytosomes was exposed in Fig. 12. The diffraction pattern of the phytosome exhibited intense and sharp crystalline peaks at 2θ of

21.42 and 30.95. The crystallinity of the optimized phytosome was found to be 49.67% and thus the extract may be molecularly dispersed in phospholipid matrix and exist in an amorphous form.

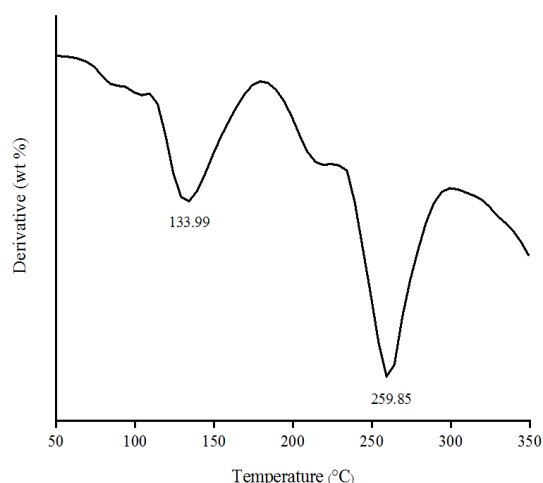


Fig. 11. Derivative weight (%) of phytosome formulation through thermogram.

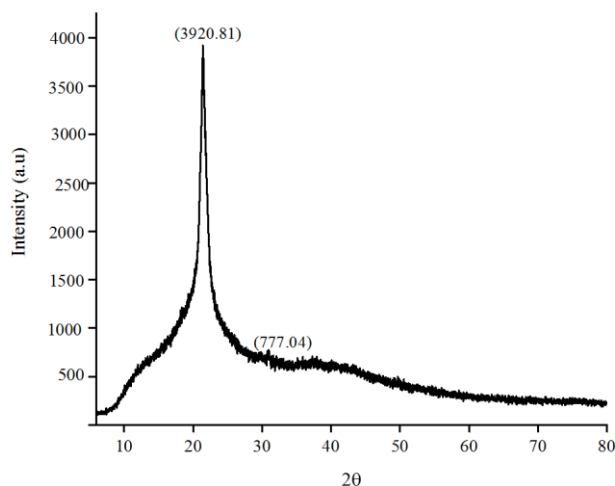


Fig. 12. XRD pattern of vasaka phytosome.

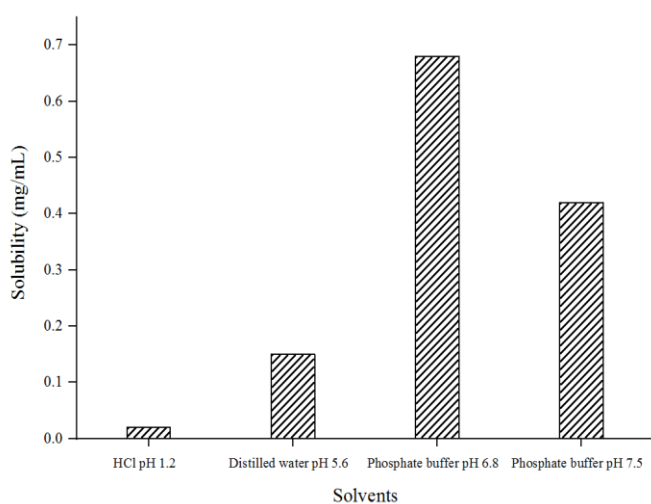


Fig. 13. Solubility of vasaka phytosome.

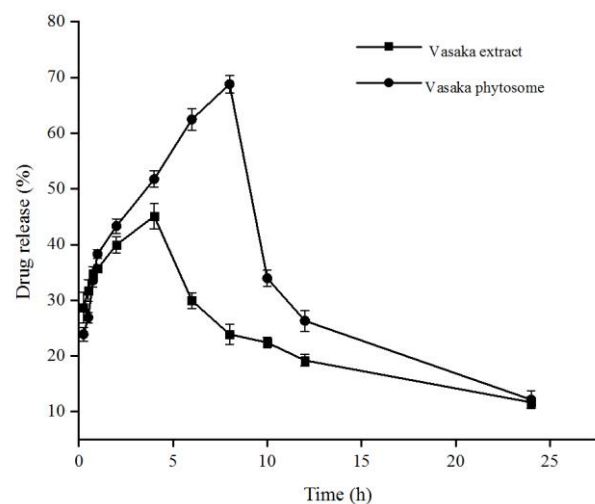


Fig. 14. *in vitro* dissolution study of vasaka extract and phytosomes.

Table 3. The saturation solubility of phytosomes

Sample	Solvents	Solubility (mg/mL)
Phytosome	HCl solution pH 1.2	0.02
	Distilled water pH 5.6	0.15
	Phosphate buffer pH 6.8	0.68
	Phosphate buffer pH 7.5	0.42

Solubility studies

In order to select the suitable media for *in vitro* release studies of vasaka phytosomes, vasicine saturation solubility in different media was determined and the results were given in Table 3 and depicted in Fig. 13. Results revealed negligible solubility of vasicine in water as well as HCl buffer of pH 1.2. Upon an increase in the pH, an increase in the vasicine saturation solubility was observed. Phosphate buffer of pH 6.8 showed an increase in the

saturation solubility when compared to pH 7.5 which showed variations in the results when the pH was shifted to 7.5. Depending on the study, a phosphate buffer of pH 6.8 was selected as the release medium for *in vitro* dissolution studies.

in vitro dissolution studies

The dissolution behaviour of vasaka extract and phytosome in phosphate buffer (pH 6.8) were correlated in Fig. 14.

The improvement in the release profile of phytosome were estimated and compared with that of extract for vasicine drug release till 24 h in dissolution media by HPLC analysis and it was found to be similar release pattern till 45 min. After this point, the extract attained a maximum of 45.08% at 4 h. In contrast, the release of vasicine from the phytosome continued to increase by reaching 68.80% at 8 h, which explained the sustained release peculiar characteristic of phytosomes. The variation in the release rate of vasicine from extract and phytosome can be associated with increased solubility and wettability of vasicine in the optimized phytosome. According to the dissolution study, the low aqueous solubility of vasicine improved by the amphiphilic nature of the phospholipon 90H and partially amorphous state of phytosome enhance the rate of dissolution to 8 h.

DISCUSSION

Phytosome, a novel emerging technique for the enhancement of bioavailability of the biomarkers by the addition of lipid molecules. Phospholipids are the potential carrier for complexing with plant active constituents in the formation of phytosomes which incorporate the molecule by intermolecular hydrogen bonding thereby increase the lipophilicity of the compounds and improve the bioavailability. Phospholipids are the main component in the formation of phytosomes which have a direct effect on the particle size (41). QbD is a new drug development paradigm in the pharmaceutical industry which aims to improve design, development, and manufacturing of high-quality products.

Pharmacokinetic profile of aqueous leaf extract of *A. vasica*, Vasa Swaras, and hard gelatin capsule encapsulating lyophilized Vasa Swaras reported the improvement of vasicine bioavailability and minimized the conversion of vasicine into vasicinone when compared to pure vasicine and marketed capsule formulation (33,42). Nanosuspension of vasicine prepared by solvent-antisolvent method enhancing the aqueous solubility and dissolution rate (43). In the earlier study, the chances of microbial contamination and storage became the critical

task to develop the final product. Lyophilization of leaf juice formed a hard cake during storage and reduces the efficacy due to absorption of moisture. To overcome the above-mentioned problem, novel technology has been implemented to develop a phytosome tablet formulation for the enhancement of oral bioavailability of vasicine. A systematic QbD approach implementing a risk assessment and design of experiment has been applied to investigate the influence of formulation parameters on the properties of phytosomes.

Based on the implementation of QbD technique for the optimization of phytosomes, the formulation containing phyto compound with a phospholipid ratio of 1:1 was found to be the optimum formulation. Box-Behnken design, a response surface methodology, was employed for statistical optimization, which involved the determination of CMA) and CQAs. The interactions of phospholipid concentration, stirring temperature, and stirring time on the responses were clearly studied and the interactions were depicted in Fig. 3. The optimized phytosome established the maximum percentage yield, minimum particle size, and the maximum entrapment efficiency. The particle size was found to be 231.0 nm and zeta potential of -23.2 mV indicated the phospholipids involved in the complex formation and enhance the stability of the optimized phytosome. The particle size of evodiamine-phospholipid complex was earlier reported as 246.1 nm showed a major role in the sustained release and enhance the oral absorption efficiency of evodiamine (44). The zeta potential with range of -20 to -30 mV was earlier demonstrated as a characteristic property of zeta potential for attraction followed by flocculation exceeding repulsion forces (45,46). The surface morphology of the optimized phytosomes showed spherical-shaped vesicle of phytosome and its average diameter was found to be 226.22 ± 31.97 nm.

Interactions of the extract, phospholipid, and phytosome complex were analyzed using FTIR and TGA conceded the weak intermolecular interaction and the characteristic exothermic peaks were identified at 133.99 and 259.85 °C. XRD reported the loss of crystallinity due to vasicine molecularly dispersed with

phospholipid matrix and may be present in an amorphous form (47). The dissolution characteristics of the extract and optimized phytosome revealed a significant enhancement of vasicine release from the phytosome when compared to extract. Accordingly, the vasicine aqueous solubility was improved by the amphiphilic nature of the phospholipid matrix and change of crystalline state to an amorphous state, which may extend the rate and dissolution to 8 h (48). The current findings showed the enhancement of vasicine bioavailability by the development of phytosome complex. This could be achieved by the use of phospholipon 90H in the complexation which increased the wettability and rate of absorption in the intestine to enhance the sustained release of the vasicine from phytosome complex.

CONCLUSION

In the current study, the QbD approach was adopted to investigate the effect of formulation variables on physicochemical properties of the vasaka-loaded phytosomes. The phytosomes were successfully developed employing thin-layer hydration technique and systematically optimized by response surface methodology strategy. The optimized phytosome formulation was characterized by SEM, TEM, FTIR, TGA, XRD analysis and *in vitro* drug release study. The rate and extent of *in vitro* vasicine release from vasaka phytosome was significantly enhanced related to vasaka extract. The study revealed that the value of phospholipids in the preparation of phytosomes with phytocompounds of low solubility to promote their solubility, absorption, bioavailability, and sustained release of the drug. This study emphasized the importance of QbD approach in the development of phytosome, a nano-based pharmaceutical formulation. Nevertheless, an *in vivo* bioavailability investigation needs to be established for affirming the application of drug delivery.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

K. Ilango contributed to concept, study design, data analysis, interpretation, manuscript editing and review. S. Nandhini contributed to literature search, experimental studies, preparation and characterization of phytosomes, data acquisition, statistical analysis, and manuscript preparation. Both the author equally contributed and approved the final manuscript.

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