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Phytosomal gel of Manjistha extract (MJE) formulated and optimized with central composite design of Quality by Design (QbD)

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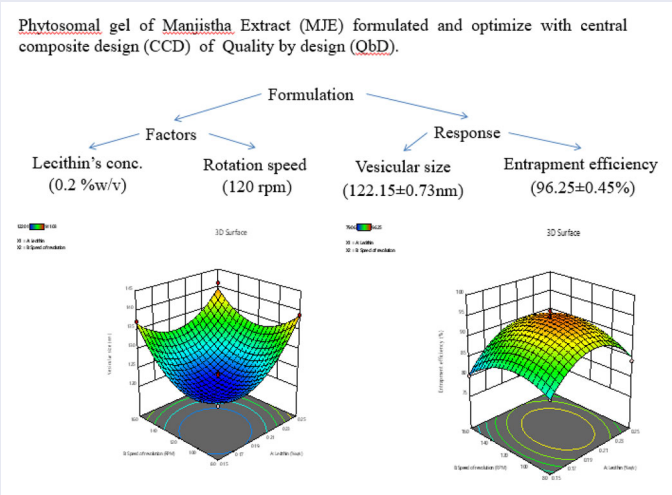
ABSTRACT

The objective of the current research to investigate the transdermal delivery of phytosomal Manjistha extract gel (MJE gel). The optimized formulation was prepared and evaluated for different parameters. MJE-loaded phytosomes were prepared by solvent evaporation method using rotary evaporator. The formulation was prepared with a variable concentration of lecithin (0.15–0.25% w/v), with stirring speed of the round bottom flask (80–160 rpm). Design expert software was used for the optimization of MJE-loaded phytosomal formulations. Central composite design-based factorial design was applied and evaluated for vesicular size and entrapment efficiency as dependent variables. The optimized formulation was further characterized with vesicle size, zeta potential, *in vitro* release. The Carbopol 934 was used to convert the phytosomal formulation (F10) into phytosomal gel and evaluated for *ex vivo* permeation to check the difference in permeation profile in compare to conventional MJE gel. The optimized MJE-loaded phytosomal formulation (F10) showed the vesicular size of 122.15 ± 3.73 nm, entrapment efficiency of 96.25 ± 2.45% with PDI value of 0.98 ± 0.06. Among the drug release kinetic models, the formulation followed the Higuchi model with drug release of 84.2 ± 4.1% in 12 h. Transmission electron micrograph showed the uniform structure and spherical shape. The prepared phytosomal gel shows prolonged release and enhanced permeation compared to conventional MJE gel.

GRAPHICAL ABSTRACT



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Entrapment efficiency; vesicular size; phytosomes; Carbopol; *in vitro* release; *ex vivo* permeation

1. Introduction

Presently, the demand for herbal medicines substantially increases and is accepted by a large population globally due to their fewer side effects and environmentally friendly nature. Herbal medicine in India and south-east Asia used as an indigenous system of medicines (Ayurveda, Unani, Siddha, Chinese, etc.). In other parts of the world, it is treated as complementary and alternative medicines. The herbal products are consumed as dietary supplements or nutraceuticals.[1] Manjistha (*Rubia cordifolia* Linn.) is a medicinal constituent present in dried roots and stem. It is a good source of anthraquinones and is reported for pharmacological properties like anti-cancer, anti-oxidant, anti-inflammatory, immunomodulatory, and hepatoprotective. It is also extensively

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used in urinary, blood, and skin disease.[2] Several bioactive compounds have been reported in root extract, including flavonoids, saponins, carboxylic acid, polyphenols, and anthraquinones (e.g., purpurin, mollugin, rubiadin, and munjistin).[3–5]

The solubility of phytoconstituents is a significant problem that affects the bioavailability. It has high molecular weight and low lipid solubility lead to poor absorption.[6–8] Poor drug absorption is a significant concern and can be overcome by several formulation approaches. Recently, the concept of phytosomes formation is widely used and reported for greater therapeutic efficacy. It is produced by forming a hydrogen bond between stoichiometric amounts of drug functionalities and phospholipids polar heads using a suitable solvent. The formation of phytosomes by hydrogen bond gives better stability over the liposome's.[9] The topical and transdermal delivery of several drugs reported for desired therapeutic effects.[10,11] To treat diseases through the skin, the enhanced drug permeation of drugs achieved through the intact epidermis and skin appendages.[12–14] The stratum corneum layer of skin for percutaneous absorption of drugs is a rate-limiting step. The development of formulation with an object to enhance drug permeation and drug retention in deeper skin layers to avoid systemic absorption. It increases the dermatological benefits by transdermal route.[15–17] New formulations development target should be effective and successful during this process, several kinds of excipients and multiple steps are required to essential quality target product profile (QTPP).[18]

The optimization of conventional drug delivery involves one variable at a one time (OVAT).[19] The formulations optimization is used to establish the "cause and effects" variable that changed simultaneously to achieve by OVAT. The partial achievement can be turned to full achievement by adopting Quality by Design (QbD) that gives a complete perception of the product and process.[20] "QbD is a systematic approach of development that begins with predefined objectives and emphasizes product, process understanding, and process control, based on sound science and quality risk management," as described in ICH Q8 (R2). According to ICH Q8 rule, the first step for applying QbD is the firm a relationship between the QTPP, critical quality attributes, critical process parameters, and critical material attributes.[21,22]

In the present study, the development of Manjistha extract (MJE) phytosome formulation by a systematic optimization approach. The optimized formulation was further characterized for drug release surface morphology and drug permeation.

2. Material and methods

2.1. Materials

Sunpure Extract Pvt. Ltd. Delhi, India, has given MJE as a gift sample and Lecithin and Carbopol 934 were obtained from Thomas Baker, Mumbai, Maharashtra, India. Also Chloroform and methanol. Other chemicals used in the formulation were of analytical grade and procured from SD Fine Chemical, Mumbai, India.

2.2. UV analysis of MJE

The standard stock solution was prepared with weighed amount of MJE (10 mg).The stock solution was dissolved separately in 10 mL of phosphate buffer saline (PBS) (pH 7.2) and 10 mL distilled water in a volumetric flask. A series of dilutions were prepared, and absorbance was measured at 215 and 225 nm, respectively, for PBS and distilled water.

2.3. Development and optimization of Manjistha-loaded phytosomes

By the thin-layer hydration method, phytosomes were prepared. First, 10 mL chloroform–methanol mixture (2:1) was taken in a round bottom flask to dissolve lecithin and cholesterol (9:1). The rotary vacuum evaporator was used to evaporate the organic solvent at 40 °C at 100 rpm for 3 min. Chloroform was evaporated and kept in a vacuum to make free it from traces of solvents. Finally, PBS of pH 7.2 was used to rehydrate the dried deposited lipid film containing 100 mg MJE for 1 h with the rotation speed of 80–160 rpm at room temperature. After that, developed dispersions were ultrasonicated for 3 min at 1 cycle/min (interval of 5 min in ice condition) to get the nanosized phytosome.[23]

2.3.1. Optimization

Design Expert 12.0.1.0, an experimental design with response surface morphology of central composite design, was used to optimize the developed phytosome. Depending upon the preliminary risk assessment, independent variables such as the concentration of lecithin (%w/v) and speed of rotation (rpm) were considered independent variables. Then the effects of these two independent variables were assessed on the vesicular size (nm) and entrapment efficiency (%) as dependent variables.[24]

2.4. Characterization of Manjistha-loaded phytosomes

2.4.1. Vesicles size distribution and vesicular size

The vesicle size and PDI evaluated the principle of dynamic light scattering by Zetasizer-1000 HS (Malvern Instruments, UK). Distilled water was used as a solvent for dilution of the sample and placed in a quartz cuvette at a 90° scattering angle.[25] All the batches were analyzed in a triplicate manner, and the mean and SD were calculated.

2.4.2. Entrapment efficiency

The ultracentrifugation method was employed to determine the entrapment efficiency of the developed phytosomes. The formulation solutions were centrifuged at 15,000 rpm at 4 °C for half an hour. The supernatants were collected and free drug concentration evaluated at 215 nm by a UV-spectrophotometer (Shimadzu-1601, Japan).[26,27] Further, the below mathematical formula was used to calculate the EE.

$$\text{Entrapment efficiency} = (\text{total drug–free drug}) \times 100/(\text{total amount of drug})$$

2.4.3. Vesicular shape

Transmission electron microscopy (TEM-Tecnai, G20, Philips Scientific, the Netherlands) was employed to study

Table 1. Independent factor's level and ranges.

	Levels and ranges		
Factors	−1	0	+1
Conc. of Lecithin (%w/v) (A)	0.15	0.20	0.25
Rotation speed (RPM) (B)	80	120	160

the vesicular shape of developed phytosomes. The sample was taken on a carbon-coated grid and stained with phosphotungstic acid. Under the microscope observed at 10–100k times enlargements at 200 kV of voltage.[20,21]

2.4.4. In vitro release studies

The dialysis membrane method studied the comparative release behavior of MJE phytosome and conventional formulation (1 gm of gel containing ∼20 mg MJE). The experiment was performed by taking 2 mL of each formulation in dialysis membrane (molecular weight cut off 12,000). The end of the bag is properly closed to avoid leakage. Separately, membrane were immersed in phosphate buffer (25 mL) as medium at fixed temperature of 37 ± 0.5 °C. The sample (5 mL) was collected at predefined time intervals such as 0, 0.5, 1, 2, 3, 4, 6, 9, and 12 h and the volume was makeup with the same fresh medium.[28] The sample was analyzed by using the UV spectrophotometer.

2.4.5. Drug kinetic modeling

Kinetics models like zero order, first order, Higuchi model, and Korsmeyer Peppas models were employed to study the release kinetics study of Manjistha from the developed phytosomal formulation, and the regression analysis was analyzed.[29]

2.5. Development of phytosomal gel

For application, the developed formulation is converted into a gel. To the above MJE dispersion weigh quantity of 0.5, 1, 1.5% w/v of Carbopol 934 was added to get uniform dispersion. The sample was stored in a dark and cool place to complete the gelling process. Triethanolamine was used to adjust the pH and to give the appearance of a clear, viscous gel. Finally, a preservative benzalkonium chloride (0.01% w/w) was added to the gel.[30]

2.6. Optimization of phytosomal gel

The prepared gel was physically examined for changes in color, pH, consistency, viscosity, and phase separation. The quantification of the drug in the gel was done by UV-spectrophotometer using a blank at 215 nm. The prepared sample, 1 g of formulation (Phytosomal gel) was dissolved in 10 mL of ethanol and evaluated for drug content in a triplicate. From the absorbance the mean drug content along with SD was calculated.[31]

2.7. Ex vivo permeation studies of phytosomal gel

Chicken egg membrane was used for the permeation study of the developed formulation.[32] The egg surface was washed with distilled water. After that, it was immersed in dilute hydrochloric acid (HCl) for 30 min. The egg membrane was removed manually, and a specific portion was cut and fixed to the diffusion cell between donor and receptor compartments with an effective area of 2.6 cm^2.[33] Phosphate buffer (pH 7.2) was used as permeation media. In the donor compartments, 1 g of phytosomal gel formulation and conventional gel (∼20 mg of MJE) were placed. The receptor compartments were maintained at the constant stirring of 100 ± 5 rpm and 37 °C. For 12 h, 1 mL aliquots from each cell were withdrawn at an interval of 0.5, 1, 2, 4, 6, 8, and 12 h. Throughout the experiment, sink conditions were maintained, and the drug in the aliquots was analyzed by UV spectroscopy at 215 nm.

2.8. Stability studies

A stability study was performed to assess the change in particle size, entrapment efficiency of the optimized phytosomal formulation and physical appearance, drug content of the optimized phytosomal gel. The formulations were sealed in glass vials and stored at a temperature of 4 ± 0.5 °C for 3 months.[34] The samples were drawn at 0, 1, 2, and 3 months in a triplicate manner.

3. Results and discussion

3.1. UV-Visible spectrophotometric analysis of MJE

The UV-visible calibration curve of MJE in phosphate buffer (pH 7.2) was found to be linear at a concentration 2–14 µg/mL ($n = 3$) with a correlation coefficient of 0.997 and in distilled water was found linear at concentration 2–14 µg/mL ($n = 3$) with a correlation coefficient of 0.998.

3.2. Preparation and optimization of Manjistha-loaded phytosomes

Phytosomes were prepared by a thin layer hydration method using lecithin and cholesterol with organic solvent chloroform and methanol. Based on preliminary studies different formulations variables, as the concentration of lecithin and rotation speed were selected as the independent factor for optimization through Design expert software. As shown in Table 1, lecithin concentration was chosen as 0.15–0.25% w/v and rotation speed (80–160 rpm). A total of 13 formulation batches were prepared, as shown in Table 2. These two independent variables were studied on the dependent variables such as vesicular size and entrapment efficiency. All the experiments were performed in a triplicate manner.

Table 2. Independent variables responses of formulation's batches.

	Factor		Response		
Formulation code	Lecithin's conc. (%w/v)	Rotation speed (RPM)	Vesicular size (nm) mean ± SD ($n = 3$)	Entrapment efficiency (%) mean ± SD ($n = 3$)	PDI (mean ± SD) ($n = 3$)
F01	0.15	80	124.95 ± 0.84	83.96 ± 0.90	0.382 ± 0.09
F02	0.25	160	138.86 ± 0.96	84.05 ± 0.66	0.453 ± 0.78
F03	0.15	80	137.15 ± 0.84	80.25 ± 0.68	0.431 ± 0.32
F04	0.25	160	141.03 ± 0.89	84.27 ± 0.96	0.198 ± 0.75
F05	0.129289	120	130.18 ± 1.22	79.06 ± 0.85	0.218 ± 0.55
F06	0.270711	120	139.95 ± 0.82	84.23 ± 0.87	0.221 ± 0.08
F07	0.2	1.58579	131.85 ± 0.71	87.85 ± 0.73	0.568 ± 0.09
F08	0.2	4.41421	138.08 ± 0.94	86.06 ± 0.77	0.668 ± 0.10
F09	0.2	120	122.03 ± 0.88	94.85 ± 1.09	0.234 ± 0.07
F10*	0.2	120	122.15 ± 0.73	96.25 ± 0.45	0.138 ± 0.06
F11	0.2	120	123.33 ± 0.54	93.78 ± 0.55	0.910 ± 0.52
F12	0.2	120	123.01 ± 0.71	93.27 ± 0.77	0.553 ± 0.34
F13	0.2	120	122.12 ± 1.03	93.85 ± 1.14	0.229 ± 0.55

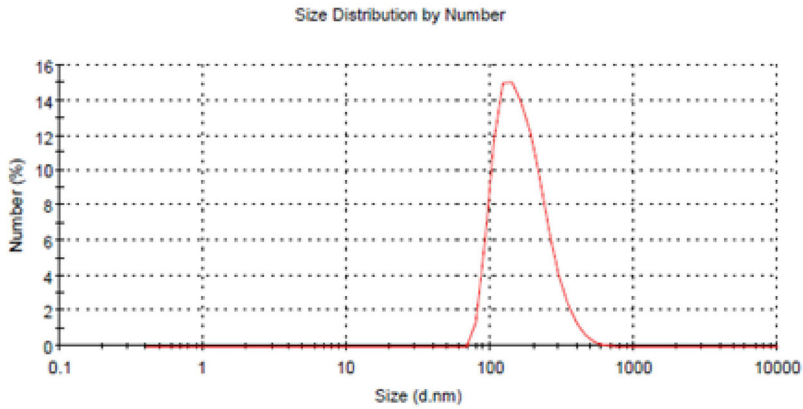

Figure 1. Particle size distribution and PDI using Zetasizer.

Table 3. The mean value of each parameter.

Parameters	Predicted value	Observed value	Standard error
Conc. of lecithin (%w/v)	0.2	0.2	–
Speed of rotation (rpm)	120	120	–
Vesicular size (nm)	122.78	122.15	0.28
Entrapment efficiency (%)	95.99	96.25	0.34

3.3. Characterization

3.3.1. Vesicle size

Vesicle size and PDI of all the batches were studied with the DLS technique, as shown in Figure 1 for optimized formulation. The mean value of each parameter along with SD is listed in Table 3. The effect of formulation variables was studied on vesicular size. ANOVA suggests that the quadratic model and F value of 129.25 ($p < 0.05$) implies the model is significant. The following coded equation describes the relation between independent factors and vesicular size:

$$\text{vesicular size} = +123.58 + 1.68A - 8.25B - 1.50AB - 8.91A^2 - 6.41B^2$$

The equation shows that factor A (lecithin concentration) has a positive effect and B (rotation speed) has established a negative effect on vesicular size. The interaction terms (AB) and the higher-order terms (A^2 and B^2) showed a negative effect on vesicular size. The variations in vesicular size with lecithin concentration and rotation speed are demonstrated by the 3D response surface and contour plot in Figure 2. Response surface plot indicates that with an increase in the concentration of lecithin, vesicular size increases, whereas by increasing the rotation speed to a certain point, vesicular size increases, but at high speed, vesicular size decreases.

3.3.2. Entrapment efficiency

The entrapment efficiency of all the batches was performed in a triplicate manner, and results are enlisted in Table 2. The effect of formulation variables was studied on the entrapment efficiency, and the results showed. ANOVA suggests a quadratic model for the design; the F value was found to be 58.26 ($p < 0.0500$). It implies the model is significant. Lack of fit F value this large could occur due to noise. Non-significant lack of fit is good, and we want the model to fit. The following coded equation describes the relation between independent factors and entrapment efficiency:

$$\text{entrapment efficiency} = +94.99 + 3.69A - 2.71B + 0.4075AB - 3.33A^2 - 2.58B^2$$

From the equation, it can be observed that Factor A (Lecithin's concentration) has a positive effect, and B (Rotation speed) negatively affects entrapment efficiency. Interaction terms (AB) show a positive effect on entrapment

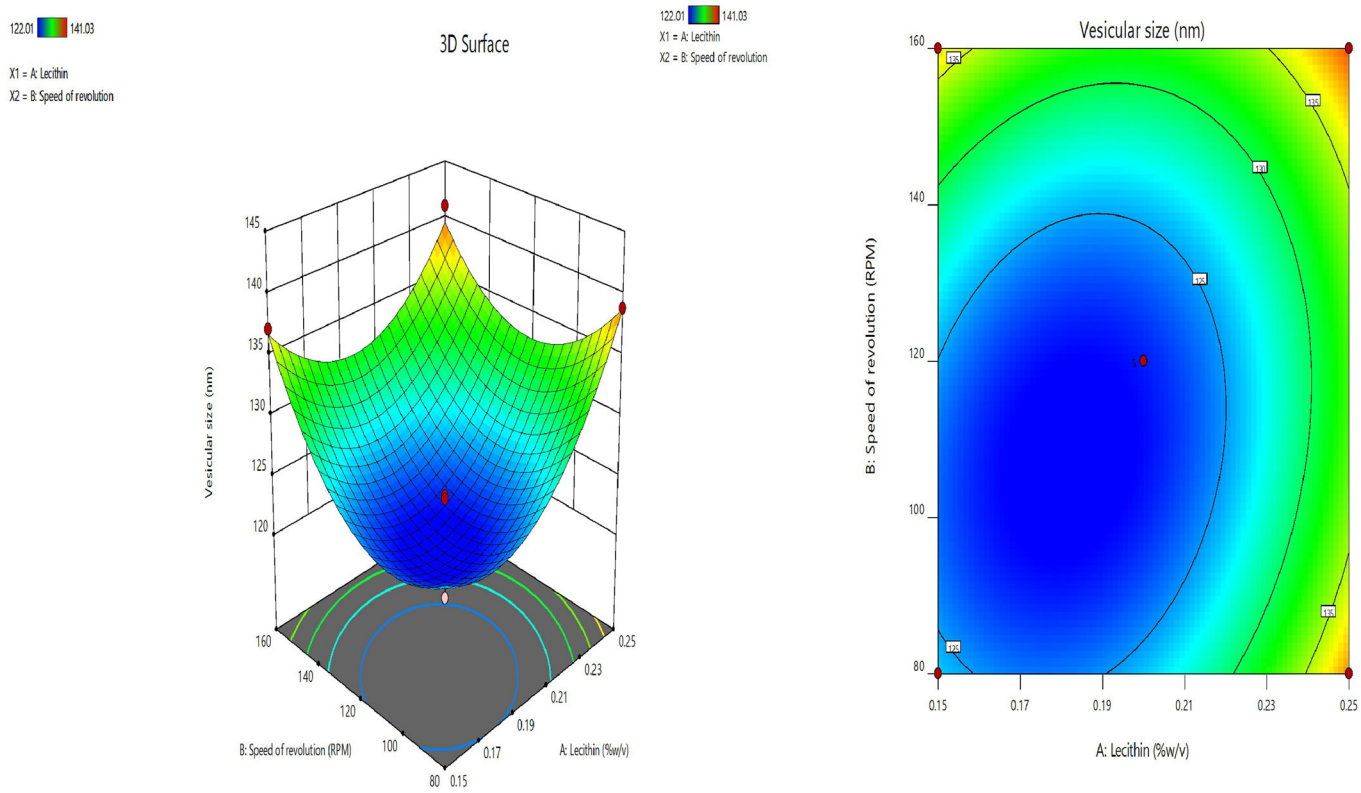

Figure 2. Effect of independent factors on the vesicular size with 3-D response surface (a) and contour plot (b).

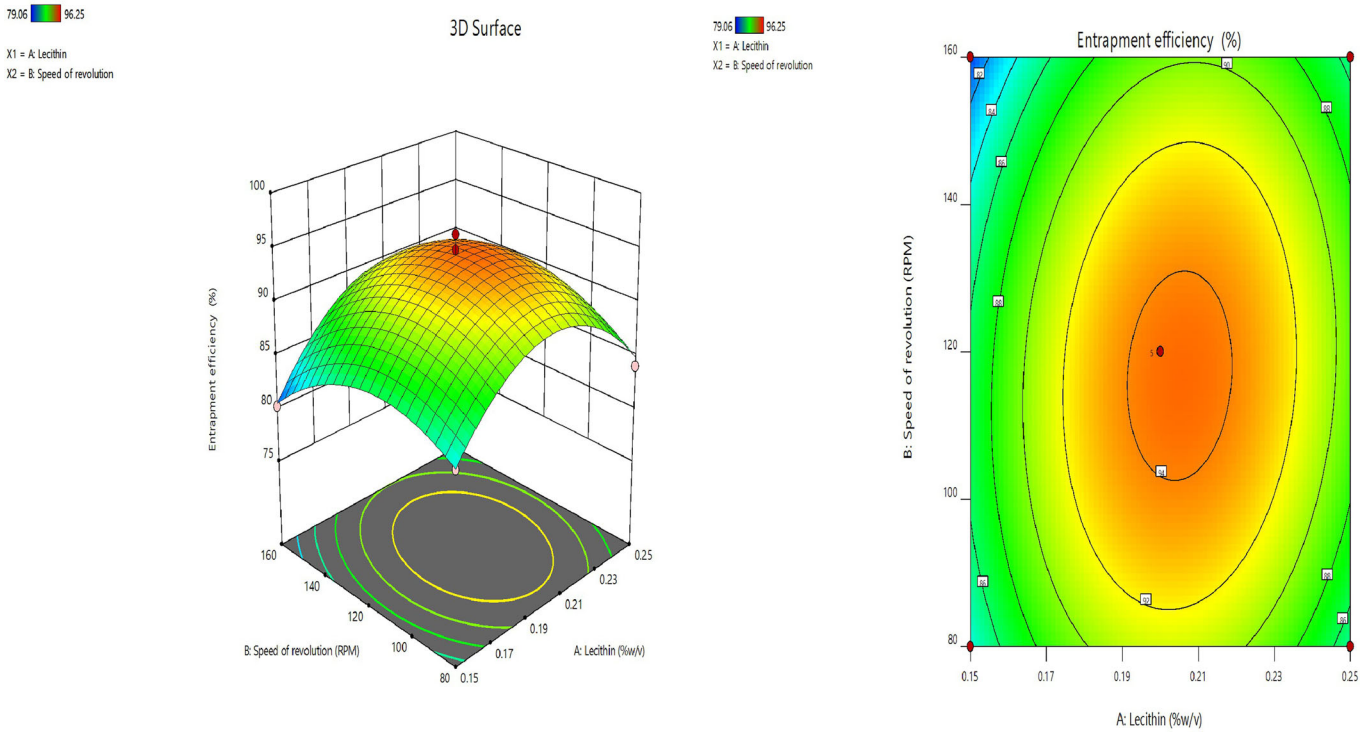

Figure 3. Effect of independent factors on entrapment efficiency with 3-D response surface (a) and contour plot (b).

efficiency, and higher-order terms (A^2 and B^2) show a negative effect. The variations in entrapment efficiency with A and B are shown by the 3D response surface and contour plot in Figure 3. Plots indicate that, by increasing the concentration of lecithin, entrapment efficiency increases. By increasing the speed of rotation to a certain point, entrapment efficiency increases, but at high speed, entrapment efficiency decreases.

3.3.3. Vesicular shape

TEM micrograph shown in Figure 4, suggests the spherical shape of the phytosomal formulation. Also, the size depicted in the TEM micrograph of optimized formulation was 122.85 and 141.77 nm, which was in correlation with the size observed in the Malvern zeta sizer.

3.3.4. In vitro release studies

To compare release behavior of optimized phytosomal formulation (F10) with the free drug solution as shown in Figure 5. The free drug solution showed 99.36 ± 4.11% release in in 6 h, whereas, with phytosomal formulation showed 84.20 ± 5.23% in 12 h. There was significant enhancement in the prolonged release was observed. An initial burst was observed in initial 2 h from optimized phytosomal formulation, probably due to the deposited drug on the phytosomal particles. [35] The entrapped extract in the lipid content able to sustained the drug release profile which is ideal for the topical formulation. An initial burst release help to reach therapeutic concentration and sustained release able to maintain the therapeutic concentration.

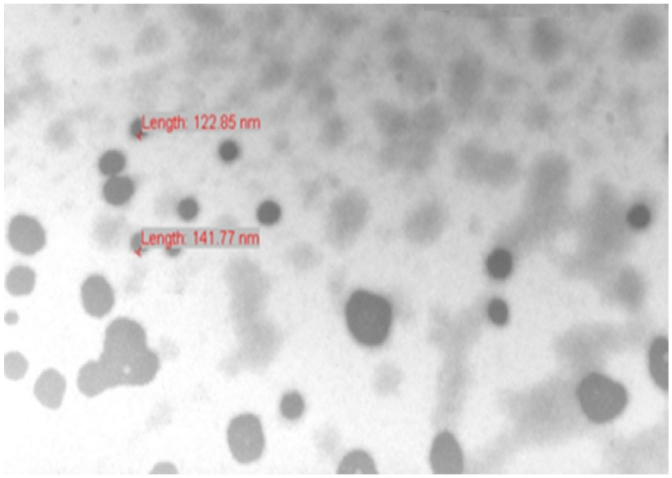

Figure 4. TEM micrograph of optimized formulation (F10).

3.3.5. Drug kinetic modeling

Data obtained by the *in vitro* release experiment was fitted to various models like the zero-order model, First-order model, Higuchi model, Korsmeyer Peppas model. The correlation coefficient (R^2) in the highest value was preferred for selecting an order of release. Table 4 shows that the highest correlation coefficient value in the case of optimized Manjistha phytosomes was found for the Higuchi matrix model, followed by first- and zero-order models. It was observed that the optimized formulation follows Higuchi's model as a best-fit model after getting the value of the correlation coefficient. The value of n was found below 0.45–0.89 (i.e., 0.62) representing that Manjistha release from optimized Manjistha phytosomes follows non-Fickian diffusion, where R^2 is the regression coefficient and K is the rate constant.

3.4. Development and optimization of phytosomal gel

Various combinations of Manjistha phytosomal gels (MJG1–MJG3) were primarily inspected for color, pH, homogeneity, viscosity, phase separation, and drug content for the development of suitable gel formulation. Many advantages of phytosome gel include enhancing dosage and promoting bioavailability of the active molecule.[36] It was found that the MJG2 (1% Carbopol gel) was transparent, of good homogeneity, consistency, and no phase separation, as

Table 4. Comparison of regression coefficients and rate constant of drug release kinetics model.

Zero-order		First-order		Higuchi model		Korsmeyer Peppas model	
R^2	K	R^2	K	R^2	K	R^2	K
0.788	1.7163	0.936	0.0042	0.902	0.0126	0.900	0.6247

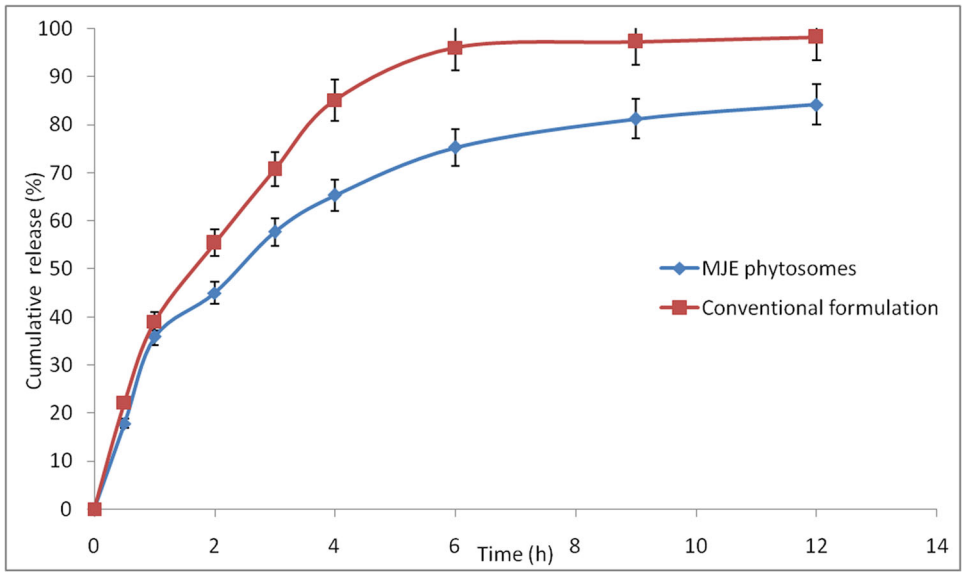

Figure 5. Comparison of in-vitro release behavior of optimized phytosomal formulation conventional formulation.

Table 5. Characterization parameters of gel.

Gel formulation code	pH (mean ± SD) ($N = 3$)	Viscosity (Pa) (mean ± SD) ($N = 3$)	Visual appearance	Drug content (%) (mean ± SD) ($N = 3$)
MG-1	6.7 ± 0.3	812.087 ± 5.23	Clear	96.98 ± 1.78
MG-2	6.65 ± 0.25	842.512 ± 6.67	Clear	97.12 ± 1.35
MG-3	6.74 ± 0.26	925.664 ± 4.89	Clear	96.52 ± 0.98

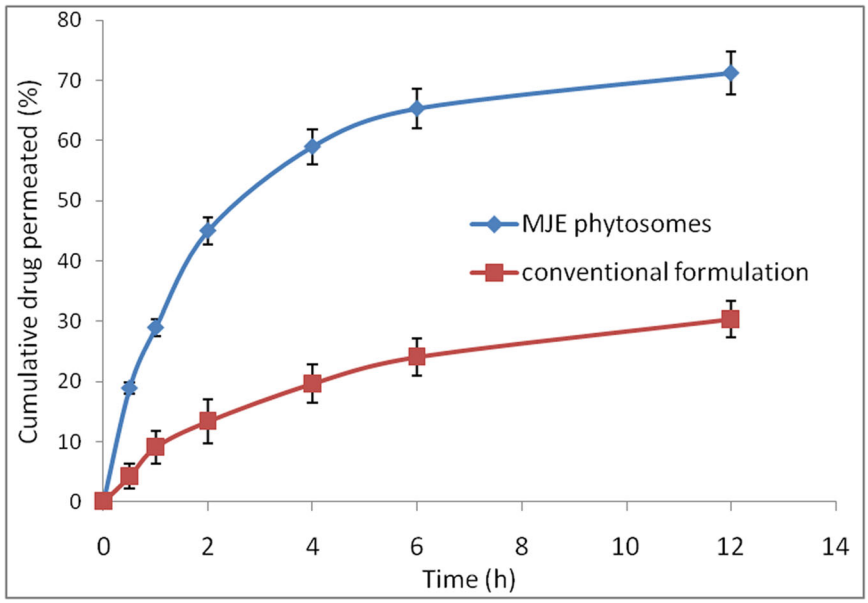

Figure 6. Comparison of permeation behavior of optimized phytosomal gel (MJG) with the conventional gel system.

Table 6. Stability analysis.

Months	Phytosomal formulation (F10)		Phytosomal gel	
	Particle size	% EE	Physical appearance	Drug content
0	122.15 ± 0.73	96.25 ± 0.45	Clear	96.25 ± 0.45
1	126.28 ± 0.24	95.17 ± 0.38	Clear	95.85 ± 0.45
2	131.78 ± 0.34	93.78 ± 0.58	Clear	94.95 ± 0.35
3	142.78 ± 0.74	91.24 ± 0.28	Clear	93.45 ± 0.25

shown in Table 5. It is safe for the skin and expects no irritation and discomfort after its application. The optimized gel was then subjected to further characterization.

3.5. Ex vivo permeation studies of phytosomal gel

Ex vivo permeation study of optimized MJG formulation and conventional gel of Manjistha across the egg membrane. Figure 6 shows the curve plotted between the calculated amount of drug permeated per unit area across the egg membrane and time. It was observed that conventional gel, cumulative drug permeated was only 31.36 ± 2.9 µg cm^{-2} in 12 h whereas, with the phytosomal gel depicted 75.20 ± 3.9 µg cm^{-2} in 12 h drug gets permeated. Also, the steady-state flux for both the formulations was calculated and found that the flux of phytosomal gel was 3.13 ± 0.33 µg cm^{-2} h^{-1} whereas, from the conventional gel, it was 1.30 ± 0.24 µg cm^{-2} h^{-1}. Thus, it can be assumed that the phytosomal gel helps increase the permeation of Manjistha across the egg membrane. Hence better therapeutics response can be expected as compared to the conventional gel system.

3.6. Stability study

The stability of any formulation during its shelf life is an important requirement, and to assure this, a stability study was performed. It was found that parameters studied for the stability study of phytosomal formulation and phytosomal gel, as shown in Table 6, were within limits. This indicates that both the formulations remained stable for 3 months. The MJE gel formulation has a good texture and clear transparent, homogeneous appearance with good characteristic odor.[37,38]

4. Conclusion

In summary, the design expert-assisted phytosomal formulation of MJE was successfully developed and optimized. A comparison of the optimized formulation with conventional formulation was performed. The *in vitro* release proved that the phytosomal formulation has shown better release. The optimized formulation was incorporated into the Carbopol gel and characterized based on pH, phase separation, viscosity, and drug content. The improved flux and permeation across the egg membrane were obtained with the phytosomal gel compared to the MJE extract-based gel. Thus, it can be concluded that the phytosomal gel of MJE is a good alternative for drug delivery. However, ahead of the need to study preclinical, clinical, and long-term stability studies.

Conflict of interest

This study reports no conflict of interest.

Acknowledgment

Thankful to Faculty of Pharmacy, Maulana Azad University, Jodhpur, Rajasthan, and Sunpure Extract Pvt. Ltd. Delhi, India.

References

[1] Gorle, A. M.; Patil, S. S. Evaluation of Anti-Oxidant and Antiacne Property of Rubia Cordifolia. Pelagia Research Library. *Der Pharm. Sin.* **2010**, *1*, 59–63.

[2] Ekor, M. The Growing Use of Herbal Medicines: Issues Relating to Adverse Reactions and Challenges in Monitoring Safety. Front. Pharmacol. **2014**, *4*, 177. DOI: 10.3389/fphar.2013.00177.

[3] Shilpa, P. N.; Venkatabalasubramanian, S.; Devaraj, S. N. Ameliorative Effect of Methanol Extract of Rubia Cordifolia in N-Nitrosodiethylamine-Induced Hepatocellular Carcinoma. Pharm. Biol. **2012**, *50*, 376–383. DOI: 10.3109/13880209.2011.608073.

[4] Chandrashekar, B. S.; Prabhakara, S.; Mohan, T.; Shabeer, D.; Bhandare, B.; Nalini, M.; Sharmila, P. S.; Meghana, D. L.; Reddy, B.; Hanumantha Rao, H. M.; et al. Characterization of *Rubia cordifolia* L. Root Extract and Its Evaluation of Cardioprotective Effect in Wistar Rat Model. *Indian J. Pharmacol.* **2018**, *50*, 12–21. DOI: 10.4103/ijp.IJP_418_17.

[5] Mishchenko, N. P.; Fedoreev, S. A.; Bryukhanov, V. M.; Zverev, Y. F.; Lampatov, V. V.; Azarova, O. V.; Shkryl', Y. N.; Chernoded, G. K. Chemical Composition and Pharmacological Activity of Anthraquinones from Rubia Cordifolia Cell Culture. Pharm. Chem. J. **2007**, *41*, 605–609. DOI: 10.1007/s11094-008-0021-1.

[6] Elnaggar, Y. S.; El-Refaie, W. M.; El-Massik, M. A.; Abdallah, O. Y. Lecithin-based nanostructured gels for skin delivery: an update on state of art and recent applications. *J. Control Release* **2014**, *180*, 10–24. DOI: 10.1016/j.jconrel.2014.02.004.

[7] Priti, L.; Heen, S. An Overview of Phytosomes as an Advanced Herbal Drug Delivery System. *Res. J. Topical Cosmet. Sci.* **2013**, *4*, 65.

[8] Armanini, D.; Nacamulli, D.; Francini-Pesenti, F.; Battagin, G.; Ragazzi, E.; Fiore, C. Glycyrrhetinic Acid, the Active Principle of Licorice, Can Reduce the Thickness of Subcutaneous Thigh Fat through Topical Application. *Steroids* **2005**, *70*, 538–542. DOI: 10.1016/j.steroids.2005.01.007.

[9] Amin, T.; Bhat, S. V. A Review of Phytosome Technology as a Novel Approach to Improve the Bioavailability of Nutraceuticals. *Int. J. Adv. Res. Technol.* **2012**, *1*, 1–15.

[10] Lu, M.; Qiu, Q.; Luo, X.; Liu, X.; Sun, J.; Wang, C.; Lin, X.; Deng, Y.; Song, Y. Phyto-Phospholipid Complexes (Phytosomes): a Novel Strategy to Improve the Bioavailability of Active Constituents. *Asian J. Pharm. Sci.* **2019**, *14*, 265–274. DOI: 10.1016/j.ajps.2018.05.011.

[11] Qumbar, M.; Ameeduzzafar, Imam, S. S.; Ali, J.; Ahmad, J.; Ali, A. Formulation and Optimization of Lacidipine Loaded Niosomal Gel for Transdermal Delivery: In-Vitro Characterization and in-Vivo Activity. *Biomed. Pharmacother.* **2017**, *93*, 255–266. DOI: 10.1016/j.biopha.2017.06.043.

[12] Pandita, A.; Sharma, P. Pharmacosomes: An Emerging Novel Vesicular Drug Delivery System for Poorly Soluble Synthetic and Herbal Drugs. *Int. Scholarly Res. Notices* **2013**, *2013*, 348186.

[13] Moon, H. J.; Ko Du, Y.; Park, M. H.; Joo, M. K.; Jeong, B. Temperature-responsive compounds as in situ gelling biomedical materials. Chem. Soc. Rev. **2012**, *41*, 4860–4883. DOI: 10.1039/c2cs35078e.

[14] Zafar, A. Development of Oral Lipid Based Nano-Formulation of Dapagliflozin: Optimization, in Vitro Characterization and Ex Vivo Intestinal Permeation Study. *J. Oleo Sci.* **2020**, *69*, 1389–1401. DOI: 10.5650/jos.ess20162.

[15] Negi, P.; Singh, B.; Sharma, G.; Beg, S.; Raza, K.; Katare, O. P. Phospholipid Microemulsion-Based Hydrogel for Enhanced Topical Delivery of Lidocaine and Prilocaine: QbD-Based Development and Evaluation. *Drug Deliv.* **2016**, *23*, 951–967. DOI: 10.3109/10717544.2014.923067.

[16] Alkilani, A. Z.; McCrudden, M. T.; Donnelly, R. F. Transdermal Drug Delivery: Innovative Pharmaceutical Developments Based on Disruption of the Barrier Properties of the *Stratum corneum*. *Pharmaceutics* **2015**, 22, *7*, 438–470. DOI: 10.3390/pharmaceutics7040438.

[17] Abd, E.; Yousef, S. A.; Pastore, M. N.; Telaprolu, K.; Mohammed, Y. H.; Namjoshi, S.; Grice, J. E.; Roberts, M. S. Skin Models for the Testing of Transdermal Drugs. *Clin. Pharmacol.* **2016**, *19*, 163–176.

[18] Jain, P.; Taleuzzaman, M.; Kala, C.; Kumar Gupta, D.; Ali, A.; Aslam, M. Quality by Design (Qbd) Assisted Development of Phytosomal Gel of Aloe Vera Extract for Topical Delivery. *J. Liposome Res.* **2020**, *19*, 1–8.

[19] Sobanko, J. F.; Miller, C. J.; Alster, T. S. Topical Anesthetics for Dermatologic Procedures: A Review. Dermatol. Surg. **2012**, *38*, 709–721. DOI: 10.1111/j.1524-4725.2011.02271.x.

[20] Arora, D.; Khurana, B.; Narang, R. K.; Nanda, S. Quality by Design (QbD) Approach for Optimization and Development of Nano Drug Delivery Systems. *Trends Drug Deliv.* **2016**, *3*, 23–32.

[21] Yu, L. X.; Amidon, G.; Khan, M. A.; Hoag, S. W.; Polli, J.; Raju, G. K.; Woodcock, J. Understanding Pharmaceutical Quality by Design. *AAPS J.* **2014**, *16*, 771–783. DOI: 10.1208/s12248-014-9598-3.

[22] Zhang, L.; Mao, S. Application of Quality by Design in the Current Drug Development. *Asian J. Pharm. Sci.* **2017**, *12*, 1–8. DOI: 10.1016/j.ajps.2016.07.006.

[23] Singh, B.; Dahiya, M.; Saharan, V.; Ahuja, N. Optimizing Drug Delivery Systems using Systematic "Design of Experiments". Part II: retrospect and Prospects. Crit. Rev. Ther. Drug Carrier Syst. **2005**, *22*, 27–106. DOI: 10.1615/CritRevTherDrugCarrierSyst.v22.i1.20.

[24] El-Gendy, N. S.; Madian, H. R.; Amr, S. S. A. Design and Optimization of a Process for Sugarcane Molasses Fermentation by *Saccharomyces cerevisiae* Using Response Surface Methodology. Int. J. Microbiol. **2013**, *2013*, 815631. DOI: 10.1155/2013/815631.

[25] Singh, B.; Kapil, R.; Nandi, M.; Ahuja, N. Developing Oral Drug Delivery Systems Using Formulation by Design: Vital Precepts, Retrospect and Prospects. *Expert Opin. Drug Deliv.* **2011**, *8*, 1341–1360. DOI: 10.1517/17425247.2011.605120.

[26] Maryana, W.; Rachmawati, H.; Mudhakir, D. Formation of Phytosome Containing Silymarin Using Thin Layer-Hydration Technique Aimed for Oral Delivery. Mater. Today Proc. **2016**, *3*, 855–866. DOI: 10.1016/j.matpr.2016.02.019.

[27] Shukla, R.; Tiwari, G.; Tiwari, R.; Rai, A. K. Formulation and Evaluation of the Topical Ethosomal Gel of Melatonin to Prevent UV Radiation. J. Cosmet. Dermatol. **2020**, *19*, 2093–2104. DOI: 10.1111/jocd.13251.

[28] Karole, S.; Gautam, G. K.; Gupta, S. Preparation and Evaluation of Phytosomes Containing Ethanolic Extract of Leaves of Bombax Ceiba for Hepatoprotective Activity. *Pharma Innov. J.* **2019**, *8*, 22–26.

[29] Sharma, S.; Sahu, A. N. Development, Characterization, and Evaluation of Hepatoprotective Effect of Abutilon Indicum and Piper Longum Phytosomes. *Pharmacogn. Res.* **2016**, *8*, 29–36. DOI: 10.4103/0974-8490.171102.

[30] El-Menshawe, S. F.; Ali, A. A.; Rabeh, M. A.; Khalil, N. M. Nanosized Soy Phytosome-Based Thermogel as Topical anti-Obesity Formulation: An Approach for Acceptable Level of

Evidence of an Effective Novel Herbal Weight Loss Product. Int. J. Nanomed. **2018**, *13*, 307–318. DOI: 10.2147/IJN.S153429.

[31] Kumar, S.; Baldi, A.; Sharma, D. K. Phytosomes: A Modernistic Approach for Novel Herbal Drug Delivery - Enhancing Bioavailability and Revealing Endless Frontier of Phytopharmaceuticals. *J. Dev. Drugs* **2020**, *9*, 2.

[32] Tari, D.; Haryan, S.; Patankar, K.; Jaiswal, V.; Samant, M.; Sivakami, S.; Dongre, P. M. A Simple Egg Membrane Model for Understanding Diffusion Characteristics of Nanoparticles and Amino Acids. *Curr. Sci.* **2017**, *112*, 1574–1578. DOI: 10.18520/cs/v112/i07/1574-1578.

[33] Mohanty, D.; Rani, M. J.; Haque, M. A.; Bakshi, V.; Jahangir, M. A.; Imam, S. S.; Gilani, S. J. Preparation and Evaluation of Transdermal Naproxen Niosomes: Formulation Optimization to Preclinical anti-Inflammatory Assessment on Murine Model. *J. Liposome Res.* **2020**, *30*, 377–387. DOI: 10.1080/08982104.2019.1652646.

[34] Sahu, A. R.; Bothara, S. B. Formulation and Evaluation of Phytosome Drug Delivery System of Boswellia Serrata Extract. *Int. J. Res. Med.* **2015**, *4*, 94–99.

[35] Iizhar, S. A.; Syed, I. A.; Satar, R.; Ansari, S. A. In Vitro Assessment of Pharmaceutical Potential of Ethosomes Entrapped with Terbinafine Hydrochloride. J. Adv. Res. **2016**, *7*, 453–461. DOI: 10.1016/j.jare.2016.03.003.

[36] Allam, A. N.; Komeil, I. A.; Abdallah, O. Y. Curcumin Phytosomal Softgel Formulation: Development, Optimization and Physicochemical Characterization. *Acta Pharm.* **2015**, *65*, 285–297. DOI: 10.1515/acph-2015-0029.

[37] Dantas, M. G.; Reis, S. A.; Damasceno, C. M.; Rolim, L. A.; Rolim-Neto, P. J.; Carvalho, F. O.; Quintans-Junior, L. J.; Almeida, J. R. Development and Evaluation of Stability of a Gel Formulation Containing the Monoterpene Borneol. *Sci. World J.* **2016**, *2016*, 7394685. DOI: 10.1155/2016/7394685.

[38] Ameeduzzafar, Qumber, M.; Alruwaili, N. K.; Bukhari, S. N. A.; Alharbi, K. S.; Imam, S. S.; Afzal, M.; Alsuwayt, B.; Mujtaba, A.; Ali, A. BBD-Based Development of Itraconazole Loaded Nanostructured Lipid Carrier for Topical Delivery: In Vitro Evaluation and Antimicrobial Assessment. *J. Pharm. Innov.* 2020, *16*, 85–98. DOI: 10.1007/s12247-019-09420-5.