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Formulation and Evaluation of Ellagic Acid and Eugenol-Loaded Phytosomes for Enhanced Skin Penetration

Satya Narayan Mohapatra,[1]* Chainesh Shah[2], Nishith K Patel[3]

1. Research Scholar, Shri JJT University, Jhunjhunu Rajasthan, India
2. Professor, Department of Pharmacy, JJT University, Jhunjhunu, Rajasthan, India
3 Professor and HOD, Pharmaceutics department, A-One Pharmacy College, Ahmedabad, Gujarat, India

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For Correspondence:

Mohapatra S. N.

Research Scholar, Shri JJT University, Jhunjhunu Rajasthan, India.

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ABSTRACT:

Phytoconstituents, such as ellagic acid and eugenol, exhibit remarkable therapeutic properties, but their limited skin penetration has been a persistent challenge in dermatological applications. This comprehensive review explores the formulation, development, and evaluation of phytosomes as a promising strategy to improve the skin permeation of these bioactive compounds. Phytosomes, phospholipid complexes of phytoconstituents, have gained considerable attention due to their ability to enhance solubility, stability, and bioavailability. This review provides insights into the various methods employed for the preparation of ellagic acid and eugenol-loaded phytosomes, emphasizing recent innovations in formulation techniques. Additionally, it discusses the factors influencing the skin penetration of phytosomal formulations, including particle size, lipid composition, and penetration enhancers. The review critically assesses in vitro and in vivo studies conducted to evaluate the efficacy of phytosomes in enhancing skin permeation, highlighting the potential benefits in the treatment of skin disorders and the development of novel cosmetic products. Furthermore, safety considerations, regulatory aspects, and future perspectives on the commercialization of phytosome-based formulations are addressed.

KEYWORDS: Phytosomes, skin penetration, bioavailability, formulation, dermatology, phytoconstituents, permeation enhancers.

INTRODUCTION [1-6]

Phytosomes (Phospholipid-drug complexes):

The phrase phytosome was created by combining the words phyto and some. "Phyto" pertains to a plant, while "some" pertains to anything resembling a cell. Over the past century, numerous plant extracts have undergone chemical and pharmacological examination to identify their chemical composition and validate their medicinal efficacy. The majority of the biologically active substances present in herbal medicines can dissolve in water, making them well-suited for use on the skin. The therapeutic efficiency of plant compounds (soluble in water) is limited because they are poorly absorbed when consumed by mouth or applied to the skin. Due to their large molecular size, which is a consequence of having numerous rings in their composition, they cannot be absorbed through passive diffusion. Due to their limited ability to dissolve in lipids and mix with other substances, they cannot enter biological membranes effectively, leading to a reduced availability of the medication. Modifiers, changes in structure, and incorporation into lipid-based vesicles can all contribute to enhancing the ability of water-soluble plant components to be absorbed by the body. A groundbreaking and patented method called Phytosomes combines plant extract standards or water-soluble phytoconstituents with phospholipid molecules to produce molecular companions that can coexist with lipids (refer to Figure 6). Due to their enhanced ability to enter the lipid-based biomembrane compared to conventional herbal extracts, lipophilic transporters provide higher availability in the body than regular herbal

extracts. Due to their strong molecular cohesion, phytosomes experience less drug leakage and possess enhanced physiological stability compared to liposomes. As a result, they are more easily absorbed by enterocytes due to their nanostructure. Phytosomes are currently used to administer nutraceuticals and herbal remedies, which is a positive development.

According to the research, Kalita et al. enhanced the solubility, permeability, and therapeutic efficacy of the resveratrol phytosomal complex compared to free resveratrol. The polymer patch with integrated drug administration was developed to assist patients in adhering to their medication schedule. Multiple experiments conducted in a controlled environment have demonstrated that the particles released from the substance maintain their original structure and can enter the hardened outer layer of the skin known as the stratum corneum. Scientists discovered that the creation of phytosomal complexes necessitates only feeble attachment and that the substances remained steady in the natural environment of drug discharge as well as while being absorbed by the skin from the patch. Because of the extended localization of phytosomal complexes in inflammatory conditions, the examination using confocal laser scanning microscopy confirms the targeting of drugs to the dermis in both short-term and long-term situations. Researchers performed an in vivo study to evaluate the anti-inflammatory effects of the enhanced formulation in comparison to the commercially accessible Diclofenac sodium gel. When applied through the skin, the combination of resveratrol in phytosomal form demonstrated potential in the test for skin irritation in rabbits and in the examination of tissue samples.

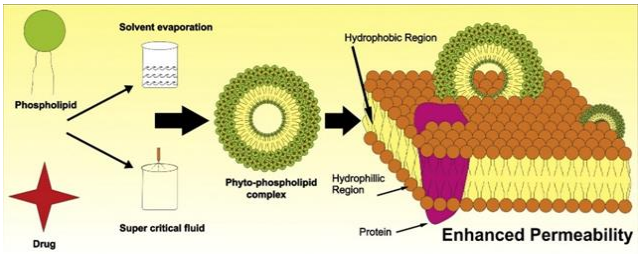


Figure 1 Advancement and infiltration of plant-derived phospholipid complexes.

Overview of Phytosomes

Phytosomes are a new type of herbal formulations that include the bioactive phytoconstituent(s) of herb extract combined with phospholipid to create lipid compatible molecular complexes. When exposed to water, these complexes form a vesicular structure. Phytosome is a recently introduced patented innovation (by Indena- An Italian pharmaceutical and nutraceutical company, in 1989) where phytomolecule create a compound with phospholipid through the formation of hydrogen bonds. The molecules have the ability to move from the aqueous phase outside of the lipid layer of the enterocyte and then enter the cell, ultimately making their way into the bloodstream. Complicated outcomes arise from the interaction of equal amounts of phospholipid with the chosen polyphenolic phytoconstituent (like basic flavonoids) in a solvent that lacks polarity. According to the physicochemical and spectroscopic information, it has been demonstrated that the primary interaction between phospholipids and substrates occurs due to the creation of hydrogen bonds between the polar parts of phospholipids (such as phosphate and ammonium groups) and the polar functional groups of the substrate. The active component (leading group) attaches to the polar head of phospholipids, becoming a fundamental component of the membrane. However, the two lengthy fatty acid chains do not participate in the formation of the complex. The two extended fatty acid chains have the ability to relocate and enclose the polar section of the compound in order to create a surface that is compatible with lipids. Moreover, when they are diluted in water, they have the ability to create tiny vesicular structures that resemble liposomes. Therefore, the term 'phytosomes' is derived from the combination of two terms: 'phyto' referring to plants, and 'some' referring to structures resembling cells. Semalty A and colleagues demonstrated the creation of a hydrogen bond between the phenolic hydroxyl group of the polyphenol component and the phosphate ion on the phosphatidylcholine component in the catechin-phosphatidylcholine complex. This was confirmed by analyzing the complex using 1H-NMR and 13C-NMR spectra. This proof suggested that the extended carbon chains encircle the active component, creating a lipid-loving covering that protects the hydrophilic part of the phospholipid and plant compound, allowing the mixture to dissolve in solvents with low polarity.

Phytosomes differ from liposomes

Phytosomes and liposomes are not the same; there are significant distinctions between a phytosome and a liposome. In terms of structure, the two are significantly different from one another, as depicted in Figure 1.1. Liposomes, in contrast to phytosomes, are created by combining a drug that is either hydrophilic or lipophilic with lipid. The drug is dispersed either in the surrounding

medium (for hydrophilic drugs) or within the layers of the membrane (for lipophilic drugs), depending on its characteristics. Unlike in phytosomes, there is no chemical bonding between the drug and phospholipid molecules in liposomes. In phytosomes, however, the active drug molecule is stabilized through the formation of a hydrogen bond between the polar head of the phospholipid and the polar functional group of the active ingredient. Moreover, within liposomes, the amount of phospholipids is five times greater compared to that found in phytosomes. The phytosome is a small group of molecules, and this distinction results in the phytosomes being more effectively assimilated compared to liposomes.

Components of phytosomes

At first, Bombardelli et al. demonstrated that the phytosomal complexes could be created by combining the active plant compound and phospholipid in a specific ratio.The literature emphasized three crucial elements required for creating phytosomes: active plant components, phospholipids, and solvents.

Standardized plant extract or active phytoconstituents

To create phytosomal complexes, one can utilize either the standardized extract from plants or the isolated active phytoconstituent, depending on their strong pharmacological effects. Mostly, the plant compounds incorporated as phytosomes consist of polyphenols that have several phenolic ring structures. These structures are too big to be taken in through simple diffusion and have limited ability to pass through the lipid-rich linings of the intestines. Compounds like hesperidin, known as polyphenols, have a preference for the water-based phase but are unable to cross the linings of the intestines. Maiti and colleagues created a new formulation of hesperetin by combining it with hydrogenated soybean phosphatidylcholine (HSPC) to address its rapid removal from the body. The compound was made using hesperetin and HSPC in equal amounts using a method that involves evaporating the solvent. The compound was subsequently analyzed for its physical and chemical characteristics in a laboratory setting, as well as its ability to combat oxidative stress in rats that were exposed to carbon tetrachloride (CCl4). The compound's behavior within the body was also studied. The research indicated that the phospholipid compound of hesperetin exhibited superior antioxidant properties compared to the unbound medication at the identical dosage (100 mg/kg), and this impact endured for an extended duration. This could potentially address the issue of the molecule being eliminated more rapidly.The researchers Kalita B et al. developed a complex of hesperidin and phospholipids in order to improve how easily it is absorbed by the body. The compound was made by boiling different amounts of hesperidin and phosphatidylcholine together. The analyzed compound was described and tested in a laboratory setting, which involved examining the release of the drug and studying its antioxidant properties.The researchers determined that the phospholipid compound of hesperidin shows potential with enhanced dissolution, leading to increased oral absorption. In comparison, certain polyphenols like curcumin and quercetin have a lipophilic characteristic, yet they are unable to dissolve in gastrointestinal fluids that are aqueous. Regarding this matter, Maiti et al. created curcumin phytosomes by combining curcumin and phosphatidylcholine in equal amounts using the solvent evaporation technique. The purpose was to address the issue of limited absorption and examine how the curcumin-phospholipid complex could protect against acute liver damage induced by carbon tetrachloride in rats. The compound offered superior safeguarding for the rat liver compared to unbound curcumin at equivalent amounts. The findings demonstrated that the curcumin-phospholipid complex exhibits enhanced hepatoprotective effects compared to free curcumin at an equivalent dosage, primarily due to its superior antioxidant characteristics. The researchers determined that flavonoids, such as curcumin, when combined with a phospholipid, exhibit greater potential for improved delivery properties and effectiveness in comparison to their pure form. Zhang and colleagues developed a complex of quercetin and phospholipids (QT-PC) to enhance its solubility and bioavailability. They also examined the potential protective effects of QT-PC against acute liver damage induced by carbon tetrachloride (CCl4) in Sprague-Dawley (SD) rats. Based on the research, it was determined that the process of forming a complex with phospholipids could be a viable method to enhance the absorption of quercetin when taken orally. This approach could also lead to stronger protective effects against acute liver damage caused by CCl4. Moreover, any present phytochemical contains a reactive hydrogen atom like –OH, -COOH, -NH, -NH2, etc. that can create a hydrogen bond with the N-(CH3) group of the phospholipid molecules. Active constituents with π electron, both water-loving and fat-loving phytochemicals, can be developed into phytosomal

complexes to enhance their ability to be absorbed by the body.

MATERIALS AND METHODS [7-14]

Part I: DEVELOPMENT AND EVALUATION OF PHOSPHATIDYLCHOLINE COMPLEXES OF ELLAGIC ACID

Development and Evaluation of Ellagic Acid – PC Complexes

Preliminary Trials:

In this phase, preliminary investigations will be conducted to assess the compatibility of ellagic acid with phosphatidylcholine (PC) and to establish a baseline for further optimization. Ellagic acid and PC will be co-dissolved in different ratios in a suitable solvent system, and the formation of complexes will be assessed through visual observation, solubility studies, and Fourier-transform infrared (FTIR) spectroscopy.

Optimization of Ellagic Acid – PC Complexes:

Based on the results of the preliminary trials, an optimization study will be conducted to determine the most suitable ratio of ellagic acid to PC. This will involve systematically varying the ratios and evaluating the complexes' characteristics, including solubility, stability, and interaction studies. The optimized ratio will be selected for further evaluations.

Evaluation of Optimized Batch of Ellagic Acid – PC Complexes:

The optimized ellagic acid – PC complexes will undergo a comprehensive evaluation to assess their quality and performance.

% Entrapment Efficiency:

The percentage of ellagic acid entrapped within the PC complexes will be determined using an established method. The formulation's ability to retain the phytoconstituent will be a key parameter for evaluation.

Particle Size:

The particle size distribution of the complexes will be measured using dynamic light scattering (DLS) to ensure uniformity and stability.

Zeta Potential:

The surface charge (zeta potential) of the complexes will be determined using electrophoretic mobility measurements, which can provide insights into their stability and dispersion behavior.

In-vitro Drug Release Study of Ellagic Acid from Ellagic Acid-PC Complexes:

In-vitro release studies will be conducted using a suitable dissolution apparatus to assess the release profile of ellagic acid from the complexes. The release kinetics and mechanism will be determined.

Part II: DEVELOPMENT AND EVALUATION OF PHOSPHATIDYLCHOLINE COMPLEXES OF EUGENOL

Development and Evaluation of Eugenol – PC Complexes

Development of Eugenol-PC Complexes:

Eugenol and PC will be combined in various ratios to develop eugenol-phosphatidylcholine complexes. The formation of these complexes will be monitored using similar techniques as in the preliminary trials for ellagic acid.

Optimization of Eugenol – PC Complexes:

The optimization process will involve statistical techniques such as regression analysis to identify the ideal ratio of eugenol to PC. Factors such as solubility, stability, and interaction will be considered.

Regression Analysis and Model Details:

Regression analysis will be performed to establish a mathematical model that correlates the ratio of eugenol to PC with complex characteristics. The model's coefficients will be determined.

Evaluation of Optimized Eugenol – PC Complex:

The eugenol-PC complexes obtained through optimization will be subjected to a series of evaluations to ensure their quality and performance.

Percentage Entrapment Efficiency (% EE):

The entrapment efficiency of eugenol within the PC complexes will be determined.

article Size:

The particle size distribution of the eugenol-PC complexes will be assessed.

Zeta Potential:

The zeta potential of the complexes will be measured to evaluate their surface charge.

In- vitro Drug Release Study of Eugenol from Eugenol-PC Complexes:

In-vitro release studies will be conducted to understand the release kinetics and mechanism of eugenol from the complexes.

This research methodology outlines the systematic approach for the development and evaluation of ellagic acid and eugenol-loaded phosphatidylcholine complexes,

ensuring a comprehensive investigation of complex formation, optimization, and characterization.

RESULTS AND DISCUSSIONS [15-20]

1.1 Part-I: DEVELOPMENT AND EVALUATION OF PHOSPHATIDYLCHOLINE COMPLEXES OF ELLAGIC ACID

1.1.1 Development and evaluation of Ellagic acid – PC complexes:

1.1.1.1 Preliminary Trials:

Result of % entrapment efficiency of preliminary batches:

Table 1: % Entrapment efficiency of preliminary batches

Batch. No.	%Entrapment efficiency (Avg. ± SD, n=3)
PT1	0
PT2	0
PT3	0
PT4	44.23 ± 2.26
PT5	47.26 ± 3.68
PT6	52.46 ± 5.36
PT7	49.52 ± 4.69
PT8	62 ± 2.36
PT9	58.37 ± 3.53

Preliminary trials indicates that there is effect of selected independent factors on the % entrapment efficiency of Ellagic acid. Literature reviews and preliminary batches results were helpful for selection of level of independent factors.

Optimization of Ellagic acid – PC complexes:

Table 2: Results of experimental design points (Average ± SD; n=3)

Run	X1(D:P)	X2(FFT)	X3(HT)	X4(Time)	Y (%EE)
EA1	0.5	40	60	75	52.06 ±2.36
EA2	1.5	40	60	75	59.36 ± 3.21
EA3	0.5	60	60	75	50.9 ± 3.69
EA4	1.5	60	60	75	58.33 ± 3.69
EA5	1	50	40	30	40.03 ± 4.92
EA6	1	50	80	30	49.5 ± 2.23
EA7	1	50	40	120	46.6 ± 3.24
EA8	1	50	80	120	55.7 ± 2.37
EA9	0.5	50	60	30	40.76 ± 4.82
EA10	1.5	50	60	30	48.7 ± 1.01
EA11	0.5	50	60	120	47.12 ± 2.61
EA12	1.5	50	60	120	55.67 ± 3.23
EA13	1	40	40	75	50.76 ± 2.13
EA14	1	60	40	75	50.08 ± 3.12
EA15	1	40	80	75	58.03 ± 1.26
EA16	1	60	80	75	59.9 ± 2.54
EA17	0.5	50	40	75	47.17 ± 3.29
EA18	1.5	50	40	75	56.8 ± 0.23
EA19	0.5	50	80	75	54.26 ± 4.30
EA20	1.5	50	80	75	62.36 ± 2.01
EA21	1	40	60	30	43.8 ± 1.36
EA22	1	60	60	30	45.2 ± 3.98
EA23	1	40	60	120	50.3 ± 1.87
EA24	1	60	60	120	51.7 ± 1.93
EA25	1	50	60	75	47.55 ± 1.65
EA26	1	50	60	75	49.89 ± 3.08
EA27	1	50	60	75	43.32 ± 0.69

Regression analysis of Y:

Objective of this study is to optimization of Ellagic acid – PC complexes using a 4 – factor, 3 - level Box - Behnken design using desirability function.

Total 27 batches were prepared and evaluated to find optimized conditions.

The data for response Y was analyzed by Design Expert 10.0.3 software.

Design Details:

Table 3: Design details

File Version	10.0.3.0
Study Type	Response Surface
Design Type	Box-Behnken
Design Model	Quadratic
Subtype	Randomized
Runs	27
Blocks	No Blocks
Build Time (ms)	171.00

ANOVA

Table 4: ANOVA for response surface reduced quadratic model

Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	878.03	8	109.75	58.41	< 0.0001	
X_1	199.68	1	199.68	106.27	< 0.0001	significant
X_2	0.27	1	0.27	0.14	0.7091	
X_3	194.49	1	194.49	103.51	< 0.0001	
X_4	127.40	1	127.40	67.81	< 0.0001	
X_1^2	96.98	1	96.98	51.61	< 0.0001	
X_2^2	80.31	1	80.31	42.74	< 0.0001	
X_3^2	84.34	1	84.34	44.89	< 0.0001	
X_4^2	49.19	1	49.19	26.18	< 0.0001	

Fit statistics of Y:

Table 5: Fit statistics of Y

Reduced Quadratic Model	
R-Squared	0.9629
Adj R-Squared	0.9464
Pred R-Squared	0.9165
Std. Dev.	1.37
Mean	50.96
C.V. %	2.69

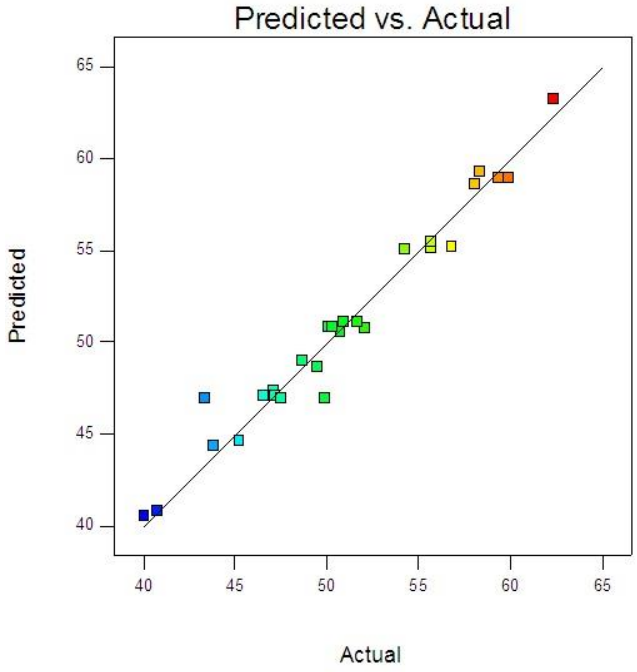


Figure 2: Predicted vs Actual plot for Y

$\%EE = + 46.92 + 4.079167 * X_1 + 0.15 * X_2 + 4.02583 * X_3 + 3.2583 * X_4 + 4.264166 * X_1^2 + 3.8804167 * X_2^2 + 3.9767 * X_3^2 - 3.037083 * X_4^2$

% EE = - 25.954 * Ellagic acid: PC ratio - 3.8654 * Film formation temperature -

0.991 * Hydration temperature +0.29737654320988 * Hydration time +17.0566 * Ellagic acid: PC ratio^2 + 0.03880416666667 * Film formation temperature^2 + 9.941E^{-003} * Hydration temperature^2 -1.499E^{-003} * Hydration time ^{2}+ 161.924

Equation 1: Polynomial equation for Y (Predictive model – code level)

Check point batches:

Table 6: Check point batches

Check point batch	Predicted	Actual (Avg. ± SD)	% Difference
1	54.9477	57.32 ± 2.76	4.317378161
2	43.4344	46.86 ± 1.83	7.88683624

Check point batches are helpful to validate polynomial equation. Results shows that there is ≥ 92% similarity between predictive and actual values.

Optimization using desirability function:

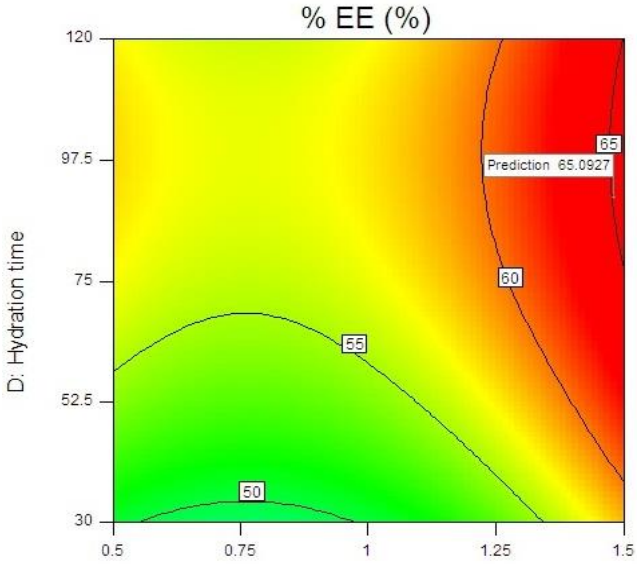


Figure 3: Contour for optimization

Table 7: Result of actual and predicted for optimization using desirability function:

X_1	X_2	X_3	X_4	Y	
				Actual	Predicted
1.480	56.591	79.605	90.694	68.73 ± 3.29	65.092

Evaluation of optimized batch of Ellagic acid – PC complexes:

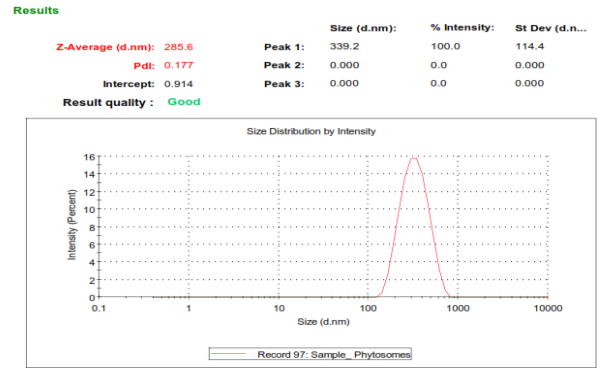
% Entrapment efficiency:

As shown in Table 7

Particle size:

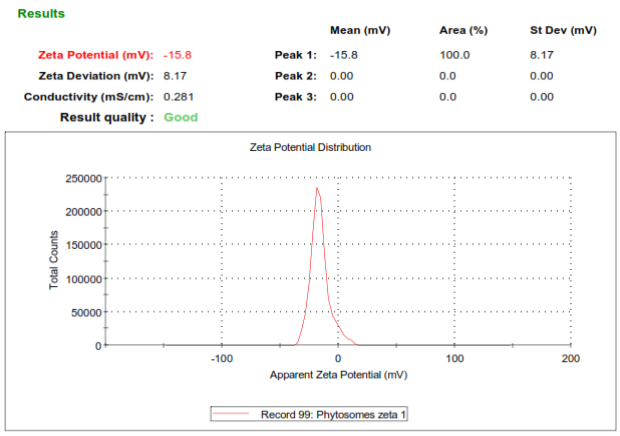
Here particle sizes were measured in the terms of average particle size diameter and the uniformity was described in the poly dispersity Index (PDI). A PDI value of 0.1–0.3 indicates a fairly narrow size distribution whereas a PDI value greater than 0.5 indicates a very broad distribution. The particle size of the optimized batch is shown in **Error! R**

eference source not found.. Average particle size of optimized batch is 285.6 nm.



Zeta potential:

Zeta potential was found to be –15.8mV. Zeta potential of the optimized batch is shown in Figure 5. In general, zeta potential value of ± 20mV is sufficient for stability suspension. In our formulation it is -15.8 mV which means it complies with requirement of zeta potential for stability.



In- vitro drug release study of Ellagic acid from Ellagic acid-PC complexes:

Table 8: Comparison of in – vitro drug release of Ellagic acid solution and Ellagic acid –PC complex:

Time (Minutes)	% Drug release of complexes (Avg. ± SD, n=3)	% Drug release of Ellagic acid solution (Avg. ± SD, n=3)
15	6.71 ± 1.4	3.68 ± 0.3
30	10.75 ± 1.84	6.75 ± 1.92
60	20.06 ± 2.41	13.49 ± 2.20
120	38.23 ± 4.82	21.39 ± 3.45
180	54.60 ± 4. 70	30.74 ± 3.30
240	70.39 ± 5.84	42.28 ± 3.95
300	84.80 ± 6.06	53.15 ± 4.52

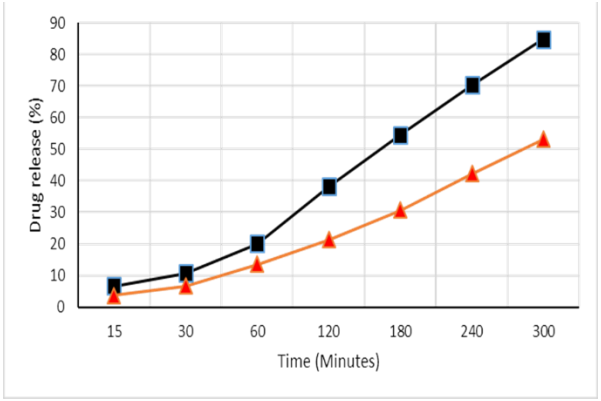


Figure 4: Comparison of % drug release of Ellagic acid – PC complexes and Ellagic acid solution

As shown in graph percentage drug release and release rate of complexes is much higher than Ellagic acid aqueous solution. This indicates that complexes of Ellagic acid may improve in vitro performance of Ellagic acid.

Developed complexes shows better in – vitro drug release profile compared to aqueous solution of Ellagic acid, which indicates optimized complexes improves permeability or absorption of drug through skin.

Part-II: DEVELOPMENT AND EVALUATION OF PHOSPHATIDYLCHOLINE COMPLEXES OF EUGENOL

Development and evaluation of Eugenol – PC complexes:

Development of Eugenol-PC complexes:

Table 9: Description of prepared batch of Eugenol-PC complexes

Process parameter and chemicals	Findings
Eugenol	20 mg
Phosphatidylcholine	100 mg
Solvent	Chloroform
Hydration by	30 ml of PBS pH 6.5
Film formation temperature	70 ºC
Hydration temperature	80 ºC
Hydration time	3 Hours

Optimization of Eugenol – PC complexes:

Table 10: Result of experimental design point (Average ± SD; n=3)

Run	X1 (D:P)	X2 (FFT)	X3 (HT)	X4 (Time)	Y (%EE)
EUG1	0.05	50	65	30	41.5804 ± 1.205
EUG2	0.05	60	65	105	53.3057 ± 1.630
EUG3	0.1	50	65	105	65.9621 ± 0.615

Run	X1 (D:P)	X2 (FFT)	X3 (HT)	X4 (Time)	Y (%EE)
EUG4	0.1	50	65	105	67.9451 ± 2.252
EUG5	0.05	50	65	180	69.8591 ± 1.730
EUG6	0.1	60	65	180	84.9295 ± 1.715
EUG7	0.05	50	50	105	65.5483 ± 1.352
EUG8	0.1	50	65	105	67.1691 ± 1.766
EUG9	0.15	40	65	105	66.8506 ± 1.932
EUG10	0.1	50	50	30	67.2553 ± 2.168
EUG11	0.05	40	65	105	56.237 ± 1.327
EUG12	0.1	60	50	105	78.9807 ± 2.018
EUG13	0.15	50	65	180	72.0805 ± 2.402
EUG14	0.05	50	80	105	61.0651 ± 1.928
EUG115	0.1	40	65	30	56.6508 ± 1.702
EUG116	0.1	50	50	180	79.8428 ± 2.213
EUG17	0.1	60	65	30	69.4969 ± 1.067
EUG18	0.1	50	80	180	74.0664 ± 1.274
EUG19	0.15	60	65	105	83.5747 ± 1.838
EUG20	0.15	50	80	105	75.7586 ± 2.170
EUG21	0.1	40	80	105	61.91 ± 1.037
EUG22	0.15	50	50	105	78.8046 ± 1.591
EUG23	0.1	60	80	105	74.1526 ± 1.581
EUG24	0.1	40	50	105	70.7902 ± 1.752
EUG25	0.1	50	80	30	62.4273 ± 1.457
EUG26	0.1	40	65	180	69.7556 ± 1.711
EUG27	0.15	50	65	30	69.8966 ± 1.652

Regression analysis of Y:

Objective of this study is to optimize of Eugenol – PC complexes using a 4 – factor, 3 - level Box - Behnken design using desirability function. Total 27 batches were prepared and evaluated to find optimized conditions. The data for response Y was analyzed by Design Expert 10.0.1 software.

Design details:

Table 11: Design details

Parameter	Description
File Version	10.0.1.0
Study Type	Response Surface
Design Type	Box-Behnken
Design Model	Quadratic
Subtype	Randomized
Runs	27
Blocks	No Blocks
Build Time (ms)	3.00

ANOVA:

Table 12: ANOVA for response surface reduced quadratic model

Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	2272.35	8	284.04	45.45	< 0.0001	
A-Eugenol:PC ratio	*822.87*	*1*	*822.87*	*131.67*	*< 0.0001*	significant
B-Film Formation Temperature	*322.88*	*1*	*322.88*	*51.66*	*< 0.0001*	
C-Hydration Temperature	*84.49*	*1*	*84.49*	*13.52*	*0.0017*	
D-Hydration Time	*577.22*	*1*	*577.22*	*92.36*	*< 0.0001*	
AB	*96.58*	*1*	*96.58*	*15.45*	*0.0010*	
AD	*170.23*	*1*	*170.23*	*27.24*	*< 0.0001*	
A^2	*61.23*	*1*	*61.23*	*9.80*	*0.0058*	
C^2	*97.94*	*1*	*97.94*	*15.67*	*0.0009*	

Fit statistics of Y:

Table 13: Fit statistics of Y

Parameter	Description
Std. Dev.	2.50
Mean	68.37
C.V. %	3.66
R-Squared	0.9528
Adj R-Squared	0.9319
Pred R-Squared	0.8692

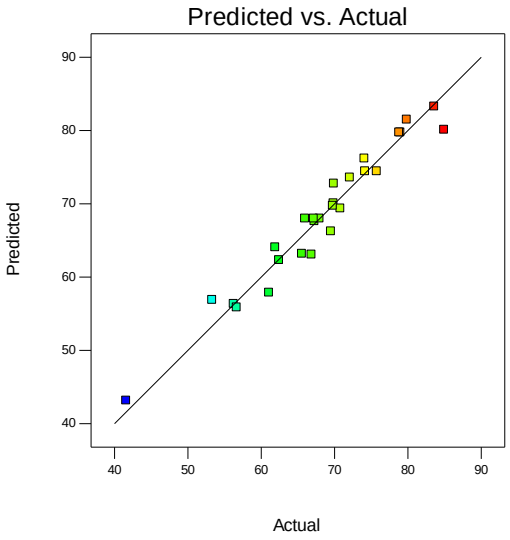


Figure 5: Predicted vs Actual plot of Y

% EE =68.0026 + 8.28084 * A + 5.18716 * B + -2.6535 * C + 6.93554 * D + 4.91387 * AB + -6.5237 * AD + -3.09316 * A^2 + 3.91194 * C^2

Equation 2: Final equation (coded factors)

% EE = +119.25109076600533 + 104.34646625582120 * Eugenol:PC ratio -0.46405718158023 * Film Formation Temperature - 2.43713294541460 * Hydration Temperature + 0.26643909601688 * Hydration Time + 9.82773277327736 * Eugenol:PC ratio * Film Formation Temperature - 1.73965212613215 * Eugenol:PC ratio *

Hydration Time - 1237.26425803500820 * Eugenol:PC ratio^2 + 0.01738640897934 * Hydration Temperature^2

Equation 3: Final equation (actual factors)

Check point batches:

Table 14: Check point batches

Check point batch	Predicted	Actual (Avg. ± SD)	% Difference
1	58.93	61.43 ± 1.74	4.24
2	76.68	74.19 ± 0.73	3.25

Check point batches are helpful to validate polynomial equation. Results shows that there is ≥ 95% similarity between predicted and actual values.

Optimization using desirability function:

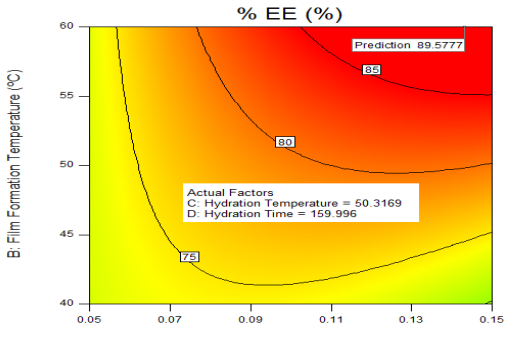


Figure 8: Contour plot for optimization

Table 15: Result of actual and predicted for optimization using desirability function

X_1	X_2	X_3	X_4	Y	
				Actual	Predicted
0.143	60	50.317	159.996	87.239 ±1.614	89.578

Optimized formula for maximum percentage entrapment efficiency was predicted by numerical optimization, this formula will be utilized for final formulation and characterization purpose.

Evaluation of optimized batch of Eugenol – PC complexes:

Percentage entrapment efficiency (% EE)

As shown in **Error! Reference source not found.**

Particle size

Here particle sizes were measured in the terms of average particle size diameter and the uniformity was described in the poly dispersity Index (PDI). poly dispersity Index (PDI) was found to be 0.173. A PDI value of 0.1 - 0.3 indicates a fairly narrow size distribution whereas a PDI value greater than 0.5 indicates a very broad distribution. Average particle size of optimized batch was found to be 203.6 nm.

The particle size and PDI of the optimized batch is shown in **Error! Reference source not found.**.

Figure 9: Particle size of optimized batch

Zeta potential

Zeta potential was found to be –8.91 mV. Zeta potential of the optimized batch is shown in

Figure 6. In general, zeta potential value of ± 20mV is sufficient for stability suspension. In our formulation, it is -8.91 mV which means it complies with the requirement of zeta potential for stability.

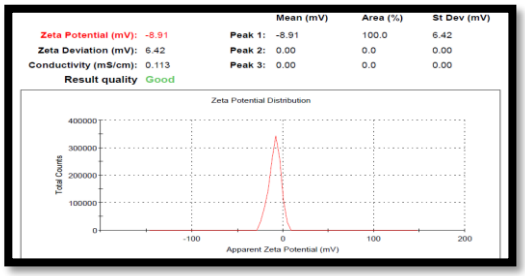
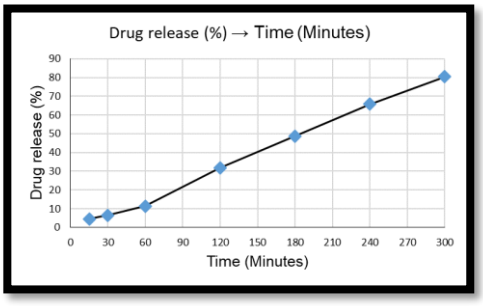


Figure 6: Zeta potential of optimized batch

In-vitro drug release of Eugenol from Eugenol-PC complexes:

Table 15: In – vitro drug release of Eugenol – PC complex



Time (Minutes)	% Drug release of complexes (Avg. ± SD, n=3)
15	4.48 ± 1.7
30	7.56 ± 1.52
60	14.35 ± 2.13
120	31.79 ± 3.18
180	48.73 ± 4.16
240	65.81 ± 4.54

300	80.43 ± 5.04

Solubility of Eugenol in water is 0.15 ± 0.22 mg/ml[179], therefore a comparison of an aqueous suspension of Eugenol with a prepared suspension of complexes was not performed.

At 300 minutes in-vitro percentage drug release of prepared complexes was 80.43 ± 5.04, which indicates a satisfactory release of Eugenol in selected media.

CONCLUSION

In this pivotal chapter, the outcomes of the experiments and evaluations carried out in the previous sections are presented and scrutinized in depth. The results are systematically organized, starting with the development and optimization of ellagic acid-phosphatidylcholine complexes, followed by the parallel process for eugenol-phosphatidylcholine complexes.

Part-I: Development and Evaluation of Phosphatidylcholine Complexes of Ellagic Acid

The chapter commences with a discussion of the preliminary trials that set the groundwork for the optimization process. Each step of the optimization journey is elaborated upon, accompanied by relevant data tables and graphs. The regression analysis of the optimized ellagic acid-phosphatidylcholine complex is detailed, highlighting the significant factors and their interactions that influence the outcome.

Moving on to the evaluation of the optimized batch, the % entrapment efficiency, particle size analysis, zeta potential measurements, and in-vitro drug release studies are meticulously presented. These evaluations provide a comprehensive picture of the behavior of ellagic acid-phosphatidylcholine phytosomes. The observations are further contextualized through discussions, comparing the outcomes with existing literature and shedding light on potential implications for the skincare industry.

Part-II: Development and Evaluation of Phosphatidylcholine Complexes of Eugenol

This section mirrors the structure of Part-I, providing an exhaustive account of the development, optimization, and evaluation of eugenol-phosphatidylcholine phytosomes. Just as in the previous section, each stage is accompanied by data representations and thorough discussions that draw connections between findings and their implications.

This chapter encapsulates the essence of the entire research journey. It commences with a concise summary of the key findings and insights derived from the comprehensive evaluations of ellagic acid and eugenol-loaded phytosomes. The research objectives set out in the initial chapters are revisited, and the extent to which they have been achieved is appraised.

The concluding remarks go beyond summarizing the empirical findings. They delve into the broader implications of the research work, considering its potential impact on the formulation of skincare products and the utilization of phytosomes in enhancing the penetration of phytoconstituents for therapeutic purposes. The significance of the contributions made by this study to the field of pharmaceutical and cosmetic sciences is highlighted, opening doors to innovative applications.

This chapter distills the essence of the research work into a comprehensive summary. It encapsulates the findings, discussions, and implications of the study, alluding to the broader significance of this research in the context of enhancing skin penetration of selected phytoconstituents using phytosomes.

The chapter concludes by restating the main conclusions drawn from the study, affirming the contributions made to the fields of pharmaceutical and cosmetic sciences. The research objectives set out in the initial chapters are revisited one final time, with an emphasis on how they have been met through meticulous methodology and analysis. This closing chapter leaves a lasting impression, reinforcing the importance of this research endeavor and its potential implications for enhancing skincare formulations and therapeutic interventions.

Implications and Applications

Expanding upon the recommendations, this chapter delves into the broader implications and potential applications of the research outcomes. The implications span multiple domains, from pharmaceuticals to cosmetics to healthcare. The chapter highlights how the findings of the study can be translated into real-world applications, fostering innovation and driving advancements in various sectors.

Contributions to Knowledge

In this chapter, the contributions made by this research work to the existing body of knowledge are systematically outlined. Each major finding, methodology, and insight is discussed in terms of its contribution to the understanding of phytosome-based formulations and their impact on skin penetration of phytoconstituents. This chapter serves as a testament to the significance of the research in advancing the scientific discourse.

Reflection on Research Process

Reflecting on the journey undertaken, this chapter provides insights into the challenges, successes, and lessons learned during the research process. It offers a glimpse into the researcher's personal growth and professional development, highlighting how the research journey has shaped their perspective and approach to scientific inquiry.

The entire research endeavor is encapsulated in a concluding synthesis. The conclusions drawn from each stage of the research are woven together to present a comprehensive understanding of the formulated phytosomes and their implications for skin penetration enhancement. The chapter concludes by reiterating the central thesis, emphasizing the significance of the research outcomes, and inviting readers to consider the broader impact of this study on the fields of pharmaceutical and cosmetic sciences.

The journey explored in "Formulation, Development, and Evaluations of Phytosomes to Improve Skin Penetration of Selected Phytoconstituents" has been one of meticulous experimentation, thoughtful analysis, and insightful conclusions. Each chapter plays a vital role in crafting a holistic narrative that not only delves into the intricacies of phytosome development but also sets the stage for future research endeavors in the realm of advanced drug delivery systems and skincare formulations.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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