Formulation and Optimization of Resveratrol-Loaded Proniosomal Gel Using 3^2 Factorial Design

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Abstract

Background: The primary goal of the study was to create and statistically improve the proniosomal gel for increased transdermal administration utilizing 3^2 factorial designs to examine the influence of both non-ionic surfactant and phospolipon to maximize entrapment efficiency (EE) and cumulative release (CR). **Methods:** Formulation of resveratrol loaded pronisomal gel was done by coaservation phase separation method. Entrapment effectiveness and drug permeation were used as dependent factors, whereas the amount of surfactant and lipid were taken as independent variables. The components were examined at three different levels (1, 0, +1), each denoting a low, medium, and high level. Nine trial runs were examined and optimized for EE and CR based on the design. To further illustrate the effects of independent and dependent factors, contour and 3D charts were produced.**Results:** EE of resveratrol was found to be in the range of 79.57-94.51%. The Model F-value of 49.48 implies the model is significant. There is only a 0.02% chance that an F-value this large could occur due to noise.P-values less than 0.0500 indicate model terms are significant. CR of resveratrol loaded proniosome was found to be in the range of 58-82%. The Model F-value of 6.73 implies the model is significant. There is only a 2.93% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. **Conclusion:** According to the study, permeation flow depends simply on lipid concentration, but entrapment efficiency is dependent on both surfactant and lipid concentration. The results suggest that Resveratrol proniosomes can act as a promising carrier.

Keywords: Resveratrol, Proniosomes, Span 60, phospholipon, 3^2 factorial designs.

1. Introduction

It is crucial to employ tools to reduce time and maximize usefulness because traditional techniques of manufacturing formulation by altering one of the variables at a time take a long time. It is crucial to comprehend the complexity of pharmaceutical formulations, and for this, well-established statistical tools like factorial design should be used. "Choosing the best element from some set of available alternatives" is how optimization is defined. It is the exercise of figuring out the best way to employ the resources that are currently accessible while taking into account every factor that influences the decisions that are made in every experiment. DoE (produce of Experiment) and other optimization approaches are used to produce high-quality formulations.[1] An experimental design, which is a statistical plan, recommends a particular set of variables. The quantity of impacts that must be calculated determines the number and layout of these design points inside the experimental region. Based on the number of components, their

levels, potential interactions, and the model's sequence, several experimental designs are used. Each experiment can be seen as a point in the experimental domain, with the definition of the point's co-ordinate being the value given to the variables in the space. [2-4]

The main obstruction to drug transport via the skin is the stratum cornea. Different approaches have been put out to get around this obstacle. One of these is the colloidal carrier, which is efficient because it can modulate the rate of release at the target site, relax the stratum corneum, which can change the barrier, and act as reservoirs for pharmaceuticals. The most popular colloidal carriers, liposome and niosome, are efficient in encapsulating both hydrophilic and hydrophobic medications. Nevertheless, stability problems with these carriers caused the recognition of proniosomes. Proniosomes are an excellent delivery system for transdermal distribution because they are a type of liquid, crystalline, compact niosomal hybrid that, when hydrated, transforms into niosomes. It also simplifies the administration, transportation, and storage of pharmaceuticals. The majority of the ingredients that go into its preparation are typically regarded as secure.[5]

Resveratrol (trans-resveratrol; trans-3,5,4'-trihydroxy-stilbene) is a polyphenolic compound from the stilbens family that is primarily found in grape skins, peanuts, and other plants used in traditional Chinese and Japanese medicine. The Polygonaceae family plant, Fallopia japonica, Polygonum cuspidatum, provided the initial source of the chemical.[6] According to claims, resveratrol has anti-aging, antioxidant, anti-inflammatory, anti-carcinogenic, and anti-obesity properties, and effects that protect the heart and brain among its health benefits. But resveratrol's utility as a nutraceutical is now constrained by its poor water solubility, high chemical instability, and low oral bioavailability. Encapsulation can improve the physical stability, chemical stability, light protection, and bioavailability of resveratrol. Because of its fast conversion into conjugated glucuronated and sufonated forms, resveratrol is a BCS class IV drug with a limited bioavailability. [7]

In the current investigation, resveratrol-loaded proniosomes were prepared and optimized using the traditional conservation phase separation method. A technique for optimization was researched to see how formulation variables affected replies. To evaluate the findings, Design Expert 13 (Version 13.0.5.0, Stat-Ease Inc., Minneapolis, MN) was used.

2. Material and methods

2.1 Materials

We received a sample of resveratrol as a gift from Meyer Organics Pvt. Ltd. in Mumbai. Another sample of phospholipon 90H and 90G was received as a gift from Meyer Organics Pvt. Ltd. in Mumbai. All experiments were conducted using the following materials: Span-20,40,60,80 (CDH Labs, New Delhi), Tween-40,60,80 (CDH Labs, New Delhi), Sodium Hydroxide Pellets extra pure, Potassium dihydrogen Orthophosphate anhydrous extra pure, Carbopol 940 (CDH Labs, New Delhi), Ethanol LR Grade (Himedia Laboratories Pvt. Ltd., Mumbai), and Distilled water. The remaining components and reagents were all of analytical grade.

2.2 Methods

2.3 Formulation of Resveratrol Loaded pronisomes

By using the traditional Co-acervation Phase Separation Method, protosomes were created [8]. The drug, lecithin, cholesterol, and surfactant (Span 60) were all consumed in a clean, dry,

amber-colored wide-mouthed glass container. Absolute alcohol (ethanol) was then added, and the container was sealed with a lid to stop the solvent from escaping. The aforementioned combination was heated on a water bath between 60°C and 700°C until the surfactant mixture entirely dissolved. When a clear solution was formed, the phosphate buffer solution (PBS; pH 7.4) was added and warmed on a water bath. Until the dispersion turned into proniosomal gel, the aforementioned combination was allowed to cool down at room temperature. In distilled water, Carbopol 940 gel 1% w/v was made, and triethanolamine was used to correct the pH. The created carbopol gel base was then gently levigated with the optimized proniosomal formulation (Opt-Pro) to create the proniosomalcarbopol hydrogel (Pro-CBP-Gel), which contains 1% resveratrol. The finished proniosomal gel was stored for later usage in the dark.[9] For comparative experiments, traditional resveratrol carbopol hydrogel (Res-CBP-Gel) and resveratrol cream (Res-Cream) with the same amount of resveratrol were also made.

2.4 Design of experiments (DoE)

The formulation was optimized and evaluated using Software Design Expert (Version 13.0.5, Stat-Ease Inc, Minneapolis, MN). The amount of surfactant and lipid were used as independent variables in a 32-randomized full factorial design, whereas entrapment effectiveness and drug permeation were used as dependent variables. The components were examined at three different levels (1, 0, +1), each denoting a low, medium, and high level. To establish an understanding of the relationship between variables and their interaction, response surface studies were performed, and contour plots and (3D) response surface plots were created. The same program was used for every statistical analysis, including analysis of variance (ANOVA). The checkpoint analysis and desirability technique were used using Design Expert software to assess potential formulation composition and create constraints for quality attributes at goal levels. A numerical optimization process utilizing the desire function was used to choose the best formulation.

To identify the primary factors and the suitable ranges in which the optima lie, primary experiments (one factor at a time method) were first carried out. Using a simultaneous optimization strategy, various excipients and medication concentrations were chosen. Based on the outcomes of the early trials, span 60 was chosen among all the nonionic surfactants. Additionally, the percentage of entrapment efficiency and percentage of cumulative release were optimized for the effects of two factors (concentrations of surfactant and lipid), three levels, and two factors. Table 1 lists the independent variables and the dependent variables used in the design.

Table 1 Coded factor

Level of Factor	**Coded Values**	**Amount of Surfactant (mg)**	**Amount of Lipid (mg)**
High	1	240	240
Medium	0	180	180
Low	-1	120	120

Table 2 3^2 Factorial design layouts

Run	**Independent variables**								
	S	**L**	**Surfactant (mg)**	**Lipid (mg)**	**Cholesterol (mg)**	**Lipid (mg)**	**Ethanol (ml)**	**Water (ml)**	**Drug (mg)**
F1	-1	-1	120	120	60	120	0.4	0.25	10
F2	0	-1	180	120	60	120	0.4	0.25	10

F3	1	-1	240	120	60	120	0.4	0.25	10
F4	-1	0	120	180	60	180	0.4	0.25	10
F5	0	0	180	180	60	180	0.4	0.25	10
F6	1	0	240	180	60	180	0.4	0.25	10
F7	-1	1	120	240	60	240	0.4	0.25	10
F8	0	1	180	240	60	240	0.4	0.25	10
F9	1	1	240	240	60	240	0.4	0.25	10

Where S= surfactant, L= Lipid

2.5 Characterization of proniosomes

a. Physical parameters

To describe the gel's color and physical state, the created gel was observed with the unaided eye. Each formula's appearance was examined, including its color, consistency, and fluidity, and each was compared to the others. According to described procedures, several physical properties like pH, spreadability, extrudability, and viscosity were also examined.

b. Vesicle size and zeta potential (surface charge analysis)[10,11]

Proniosomal gel (100 mg) was placed in a tiny ambar-colored glass vial, and double-distilled water was added, sometimes shaking the vial for 10 minutes. Following hydration, the size, polydispersity index, and zeta potential of the niosome dispersion were evaluated using photon correlation spectroscopy on the Zetasizer Nano ZS from Horiba at a scattering angle of 90° and a temperature of 25°. Results represent the means of experiments conducted in triplicate.

c. Entrapment Efficiency[12]

The centrifugation method was used to determine entrapment efficiency. Proniosome gel was thoroughly moistened with 10 cc of pH 7.4 phosphate buffer before being bath sonicated for roughly 10 minutes. Niosome-containing aqueous dispersion was separated from medication that wasn't captured by centrifugation at 15000 rpm and 40C for 30 minutes. The pellets were once more washed with 10 ml of pH 7.4 phosphate buffer after the supernatant was separated. The two supernatants were then combined, and the amount of medication in each was measured spectrophotometrically at 305 nm. The results of each spectrophotometric analysis were averaged after being performed in triplicate. Entrapment effectiveness was calculated using the formula below.

$$\%\ \text{Entrapment efficiency} = \frac{\text{Amount entrapped}}{\text{Total amount}} X100$$

d. Drug Content[15]

100 mg of the best proniosomal gel were precisely weighed and then transferred to a 50 mg volumetric flask. The gel was then dissolved, and methanol was used to bring the volume up to the mark. By utilizing methanol as a blank and UV visible spectrophotometry at 305 nm to measure the absorbances. if necessary, appropriately diluted. Prior to analysis, the placebo interference was verified. A calibration curve was used to determine the drug content percentage. Three duplicates of the experiment were performed.

e. In-vitro Release Study[13]

Dialysis tubing was used to measure the drug's release from proniosomes. The pH 7.4 phosphate solution was left on the dialysis membrane for 24 hours. In order to activate before use. The suspension equivalent to 10 mg of resveratrol was taken in a dialysis bag, which was then placed in a beaker with 75ml of 7:3 pH 7.4 phosphate: Ethanol. Proniosome gel was then hydrated with pH 7.4 phosphate. The beaker was put over a magnetic stirrer that stirred at a speed of 100 rpm while maintaining a 3710C temperature. At predefined intervals (0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 h), 5 ml aliquots were removed and fresh diffusion media was added in their stead. After being adequately diluted, the withdrew samples were examined for drug content using a UV spectrophotometer at 304 nm while holding the diffusion medium as a control. Values were averaged after each decision was made twice.

The optimized pronisomal dispersions (Opt-Pro), proniosomalcarbopol hydrogel (Pro-CBP-Gel), conventional plain resveratrol containing carbopol hydrogel (Res-CBP-Gel), and conventional cream of resveratrol (Res-cream) were used in an in-vitro release study with formulations equivalent to 10 mg of drug.

Table 13 Protocol of in-vitro study using Dialysis Tubing Method

Membrane used	**Cellophane membrane (60µm)**
Temperature	$37\pm1^{0}C$
Dissolution medium	7:3 pH 7.4 phosphate : Ethanol
Total volume	75 ml
Replacement volume	5 ml

f. Data Analysis via Drug Release Kinetics study[14]

The mathematical models zero-order kinetics, first-order kinetics, higuchi kinetics, and korsmeyer-peppas kinetic were used to determine the mechanism of resveratrol release from proniosomal formulations. The graphs of cumulative release against time, log cumulative release against time, cumulative release against square root of time, and cumulative release against log time were also plotted.

g. Stability Study [16]

The stability testing defines a shelf life for the drug product and suggests suggested storage conditions. It also shows how the quality of a drug product changes over time under the effect of several environmental elements, including temperature, humidity, and light. Testing of the characteristics of the drug product that are prone to change during storage and are likely to affect quality, safety, and/or efficacy should be a part of stability studies. The examination must to include both chemical and physical characteristics.

Physical stability

Optimized batch was evaluated for physical parameters like pH, Phase separation, change in colour, odour. Physical stability testing was done by visual inspection,

Chemical stability

In order to conduct chemical stability investigations, the improved Pro-CBP-Gel was stored for six months at two different temperatures: ambient temperature (25°0.5°C) and refrigerated

temperature (2°-8°C). Proniosomal formulations were kept in amber-colored glass bottles for the duration of the trial. Over the course of 1, 3, and 6 months, samples were taken out at various intervals and examined for drug concentration, vesicle size, zeta potential, viscosity, spreadability, and entrapment effectiveness.

3 Result and discussion

3.1 Optimization of formulation variables

The formulations were optimized by considering the parameters like maximum entrapment efficiency and smaller particle size. Nine runs performed for the response surface methodology based on the 3^2design. Based on the DoE, the factor combinations yielded different responses as presented in table 3. Analysis of variance (ANOVA), regression coefficients, regression equation and Mathematical relationships were generated using multiple linear regression analysis for the mentioned variables as shown in table 1.

Table 3 Formulation table by factorial design

Run	Independent variables		% Entrapment efficiency	% Cumulative drug release at 24 hrs
	Surfactant	Lipid		
F1	120	120	91.93	63
F2	180	120	93.61	74
F3	240	120	94.51	82
F4	120	180	84.07	59.28
F5	180	180	86.02	55.26
F6	240	180	91.47	62
F7	120	240	79.57	56.26
F8	180	240	83.26	58
F9	240	240	86.93	61

The % EE of resveratrol was found to be in the range of 79.57-94.51% as shown in table 3.The **Model F-value** of 49.48 implies the model is significant. There is only a 0.02% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. Thus, both the factors significantly affect the entrapment efficiency of Resveratrol. For percentage entrapment efficiency polynomial equation in terms of coded factors was obtained as-

$EE=+87.93+2.89^{*}A-5.05^{*}B$

Where A= Surfactant concentration; B= Lipid concentration

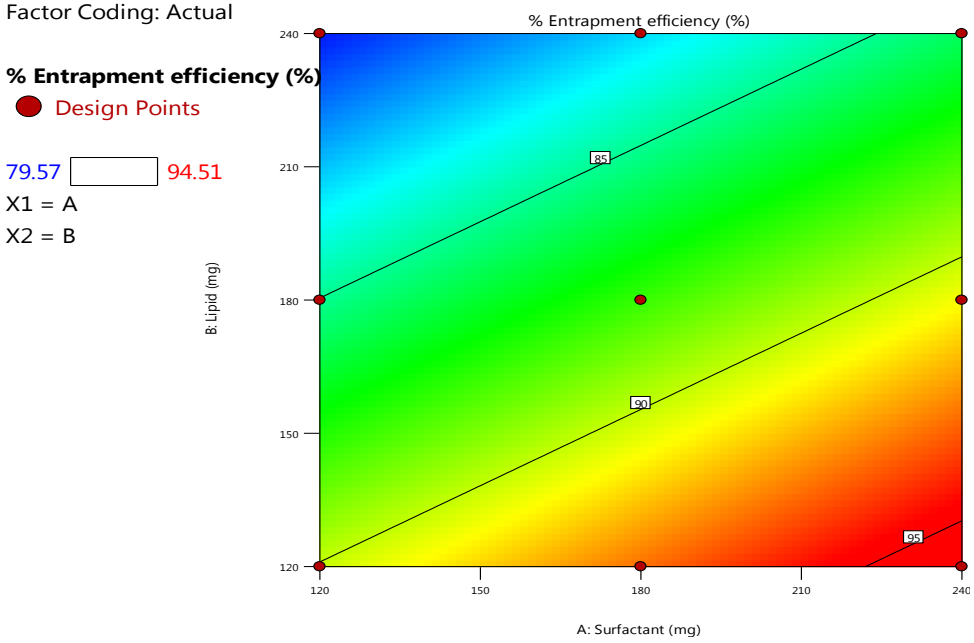

Figure 1 Contour plot of percentage entrapment efficiency

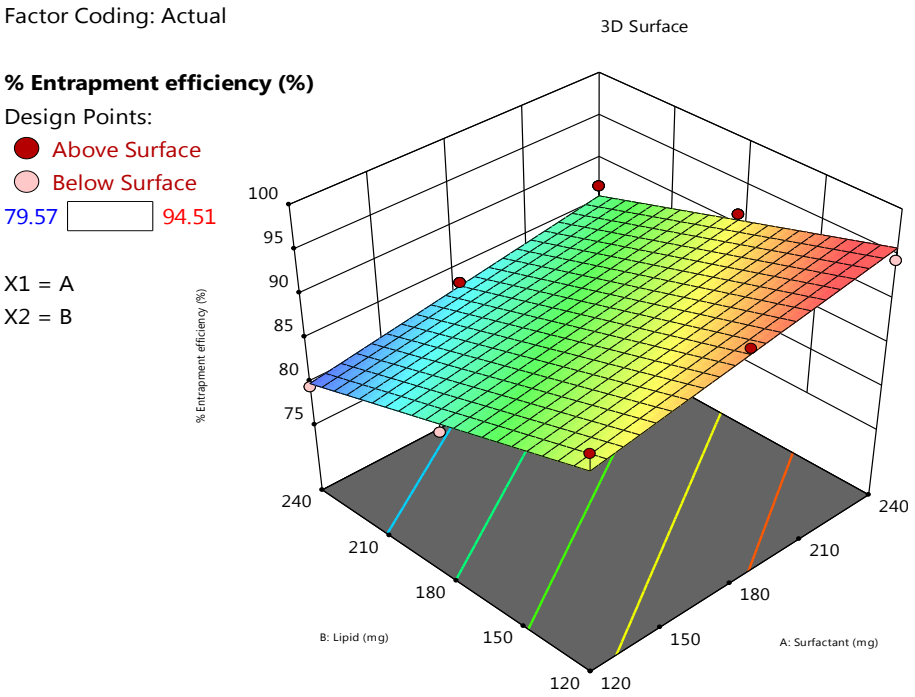

Figure 2 3D surface graph of percentage entrapment efficiency

The % CR of resveratrol loaded proniosomes was found to be in the range of 55.26-82 % as shown in table 3. The **Model F-value** of 6.73 implies the model is significant. There is only a 2.93% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model

reduction may improve your model. For percentage cumulative release polynomial equation in terms of coded factors was obtained as-

% CR=+63.42+4.41*A-7.29B

Where A= Surfactant concentration; B= Lipid concentration

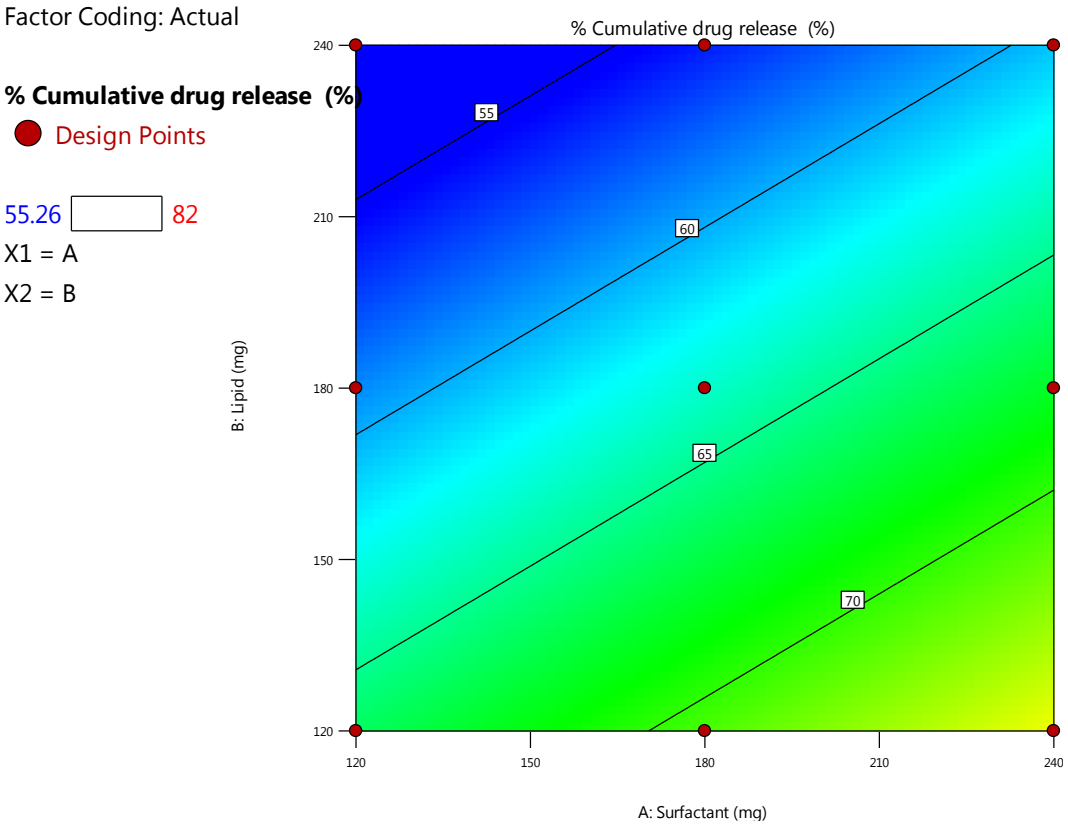

Figure 3 Contour plot of percentage Cumulative Release

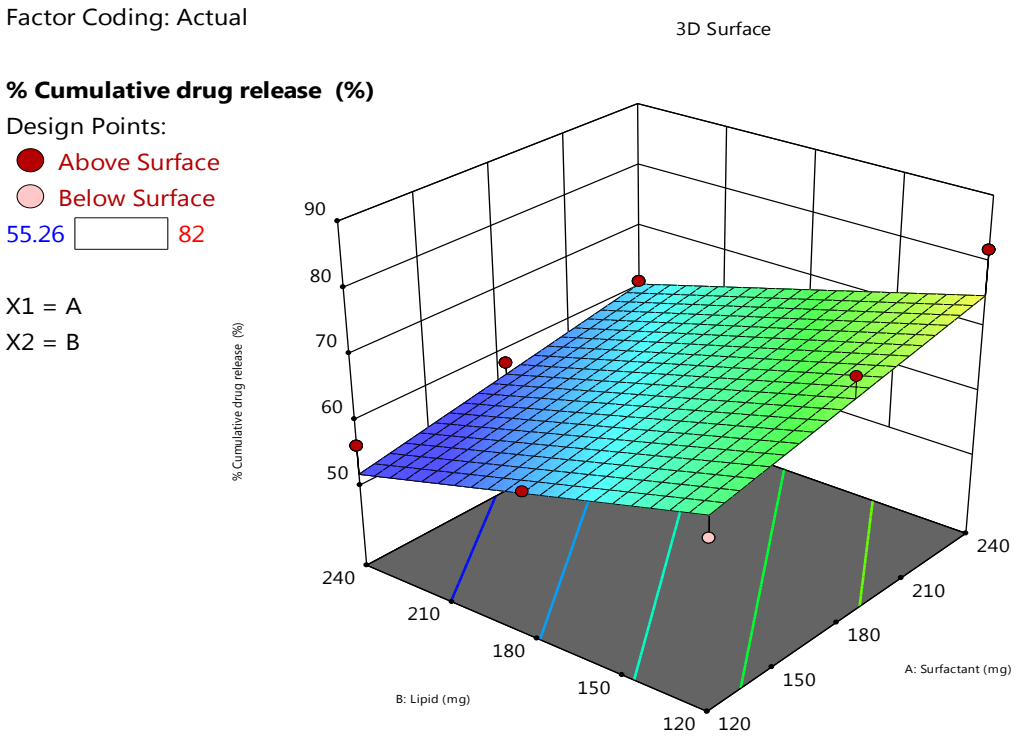

Figure 4 3D of percentage Cumulative Release

3.2 Optimization and Confirmation of DoE

A numerical optimization technique using the desirability approach was employed to prepare maltodextrin based doxorubicin HCl Proniosomes with the desired responses.

Many batches were suggested by the software as the optimized batch i.e. any of the combination would be taken will give maximum percentage entrapment efficiency and maximum drug release. Out of which four batches were selected with maximum desirability.

Formulation code	Surfactant concentration	Lipid concentration	% Entrapment efficiency	% Cumulative release	Desirability
F1	240	120	95.868	75.122	1
F2	230.948	126.010	94.927	73.727	1
F3	228.899	126.724	94.768	73.489	1
F4	217.178	124.908	94.356	72.84	1

All the batches were formulated and entrapment & release were checked manually. F1 manual entrapment and release were found to be 96.67% and 76.88% respectively which was close to the values given by software i.e. 95.868% and 75. 122% respectively. Hence it was considered as the optimized batch for further evaluation.

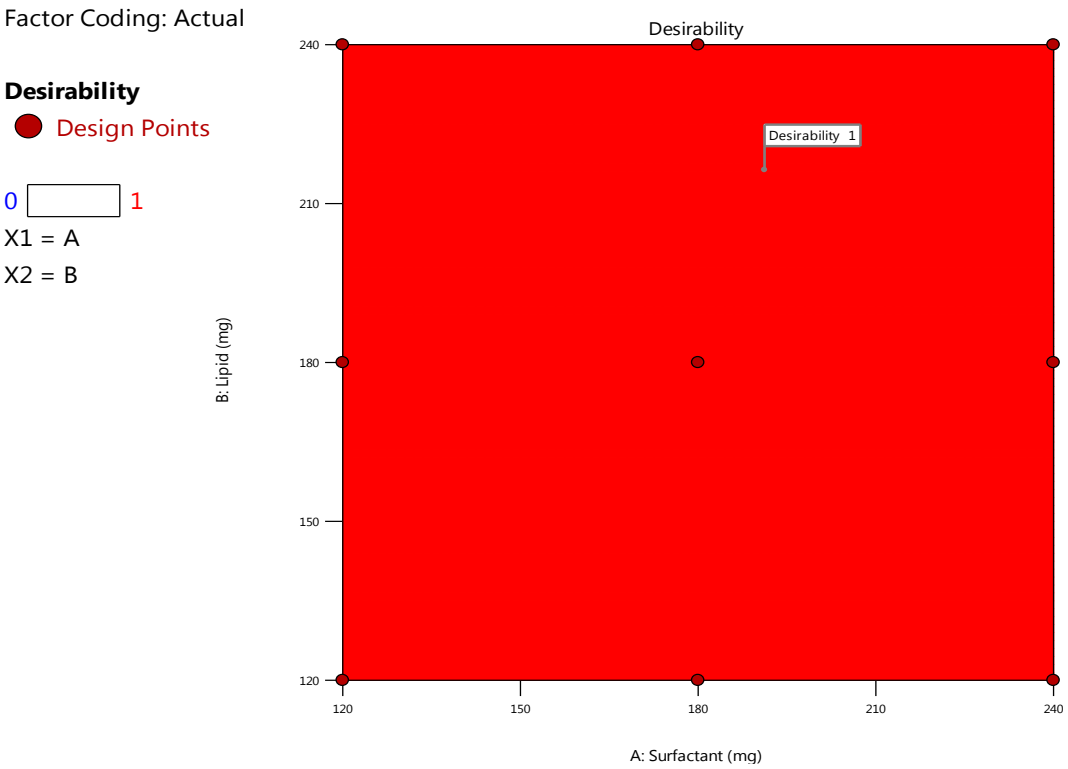

Figure 5 Contour Plot of desirability of the optimized batch from the software

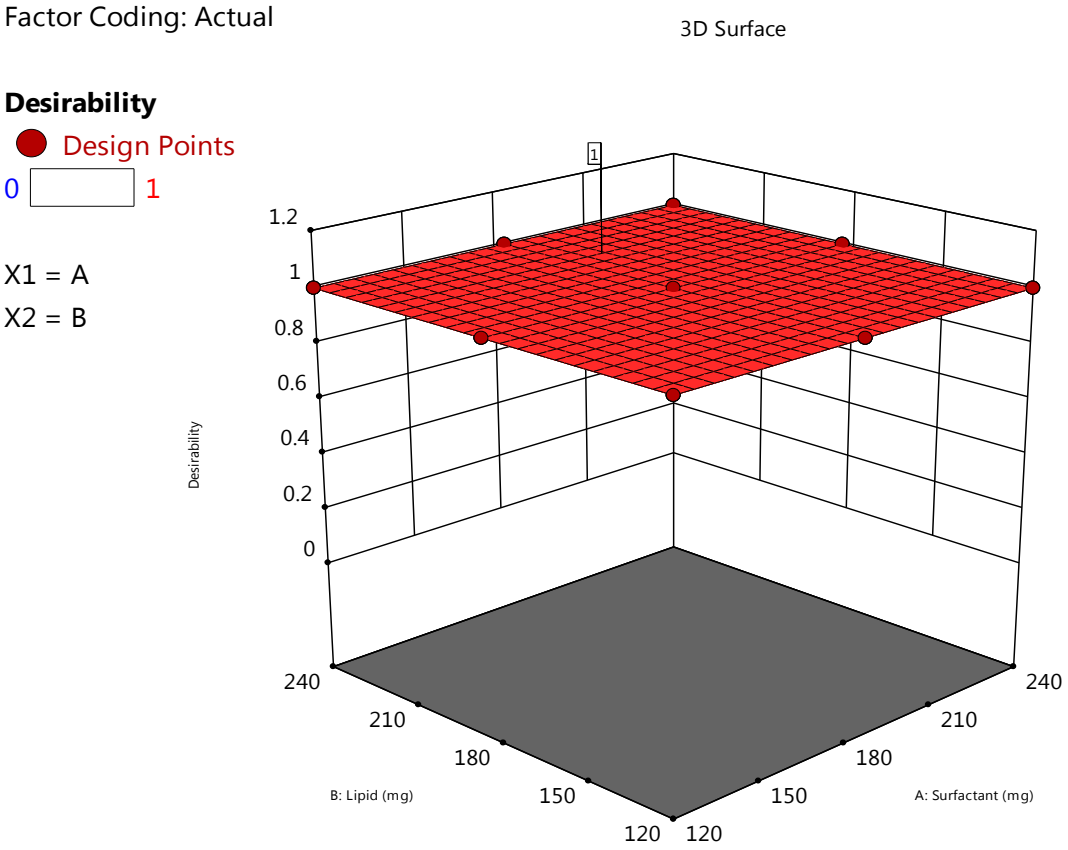

Figure 6 3D Plot of desirability of the optimized batch from the software

3.3 Physical parameters

Table shows the physical parameters and obervations of Pro-CBP-Gel.

Table 4 Physical evaluation parameters

Physical Parameters	Observations
Color	White gel
Homogeneity	Homogeneous
Odor	No smell
Phase separation	No phase separation
Grittiness	No grittiness
pH	6.76
Spreadability	13.67gm cm/sec
Viscosity	25000cps
Extrudability	15.31 g/cm^2

3.4 Vesicle size and zeta potential

Vesicle size of Pro-CBP-Gel was found out to be 331.7 nm. Polydispersity index was also provided automatically by the instrument and it was 0.458 which indicates broad particle size distribution. The zeta potential of the optimized batch was found to be -55.9 mV which proves that vesicles had strong negative charge which concludes that vesicles repel each other. And hence they will not aggregate or coalesce.

3.5 Entrapment Efficiency

As shown in table 3, all the formulation batches showed very good entrapment efficiency. Resveratrol is a hydrophobic drug and must be entrapped within the hydrophobic tail of noisome. Batch containing 240mg surfactant span 60 and 120mg of phospholipon 90H showed maximum entrapment efficiency because the drug leaching from vesicle is low due to high phase transition temperature and low permeability of surfactant.

3.6 In-vitro release study

In vitro release study of different resveratrol formulations were performed by dialysis tubing method using different diffusion medium. The results obtained are listed in table

Table 5 In vitro release data for various Resveratrol formulations

Time in hrs	Opt-Pro	Pro-CBP-Gel	Res-CBP-Gel	Res-cream
0.25	1.98	2.67	1.16	0.0682
0.5	3.73	4.56	1.98	0.8122
1	12.01	14.06	6.96	2.8943
2	16.92	19.76	9.67	4.7853
3	24.11	27.84	14.24	5.7823
4	30.88	35.72	19.28	9.9983

5	39.76	43.99	25.26	11.9989
6	45.99	49.68	31.89	17.2100
7	52.52	57.37	38.76	20.1383
8	58.01	63.39	44.9	22.7641
10	62.78	72.26	49.67	27.4517
24	76.88	89.56	55.12	42.88

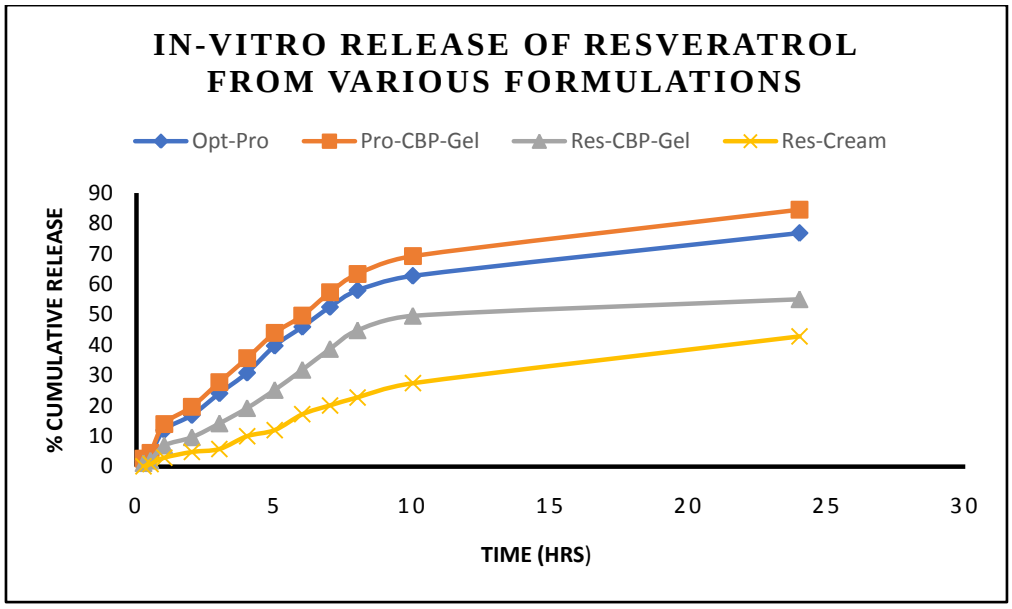

Figure 7 In-vitro release of resveratrol from various formulations

From above release study it was observed that maximum release is obtained in Pro-CBP-Gel.

3.7 Data Analysis via Drug Release Kinetics study

Mathematical modelling of the release profile of optimized batch was done using zero order, first order, Higuchi and Korsemeyers Peppas model to understand the kinetics of Resveratrol release from proniosomal gel. The results of model fittings as analyzed employing different mathematical models are presented in Table 6.

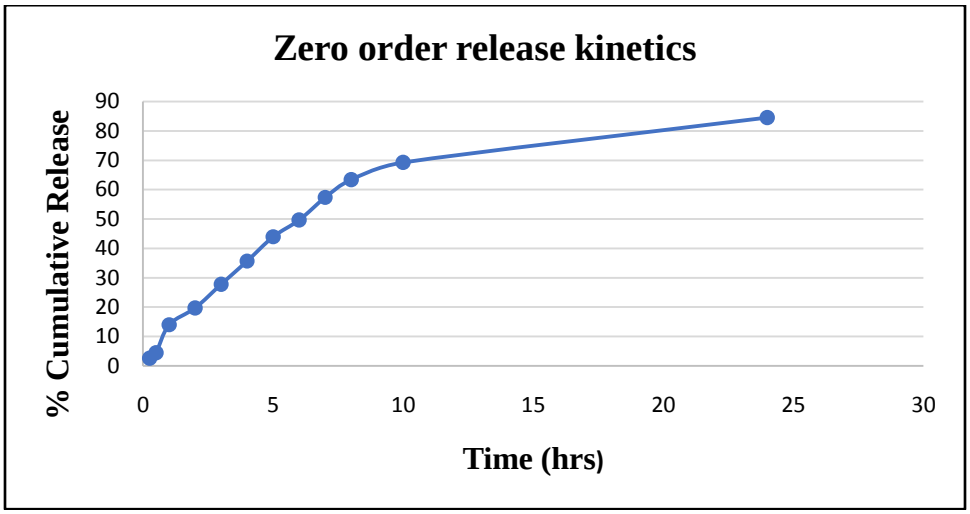

Figure 8 In vitro % cumulative release verses time of sample withdrawal graph (Zero order) of Pro-CBP-Gel

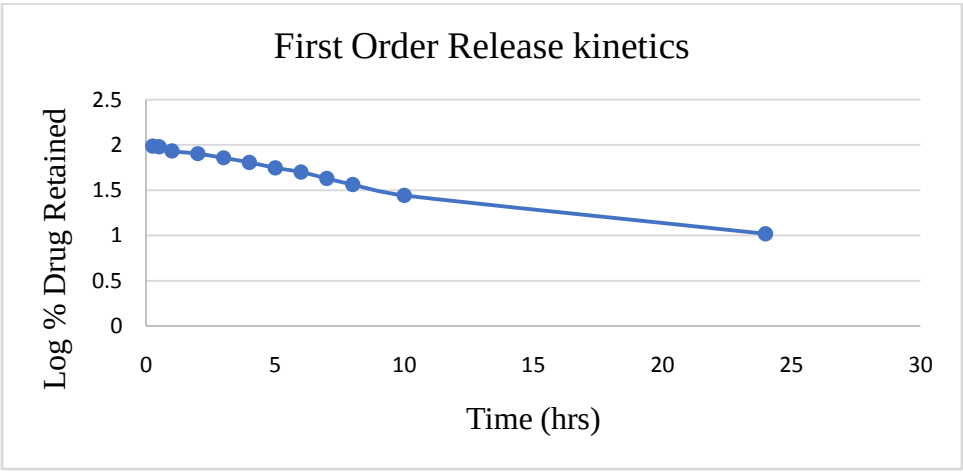

Figure 9 In vitro log % Drug retained verses time graph (First order) of from Pro-CBP-Gel

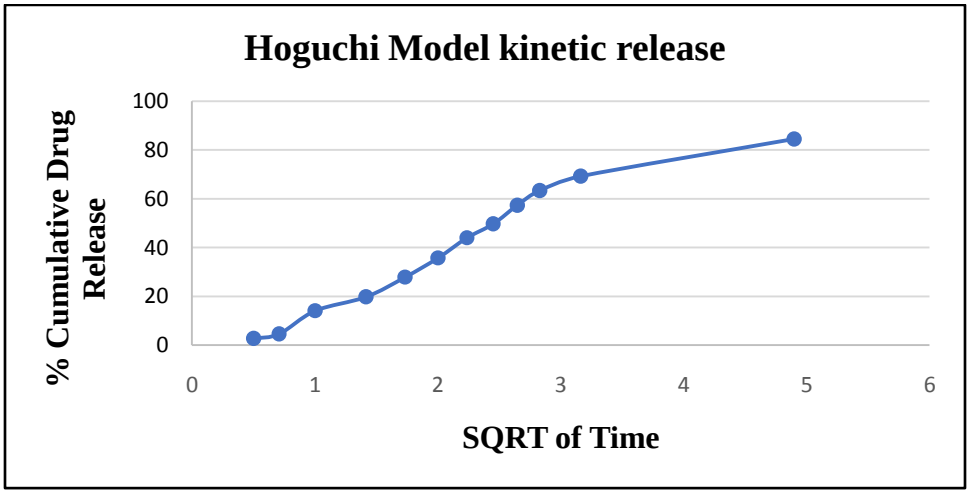

Figure 10 28In vitro % cumulative drug release verses square root of time graph (Higuchi release) of Pro-CBP-Gel

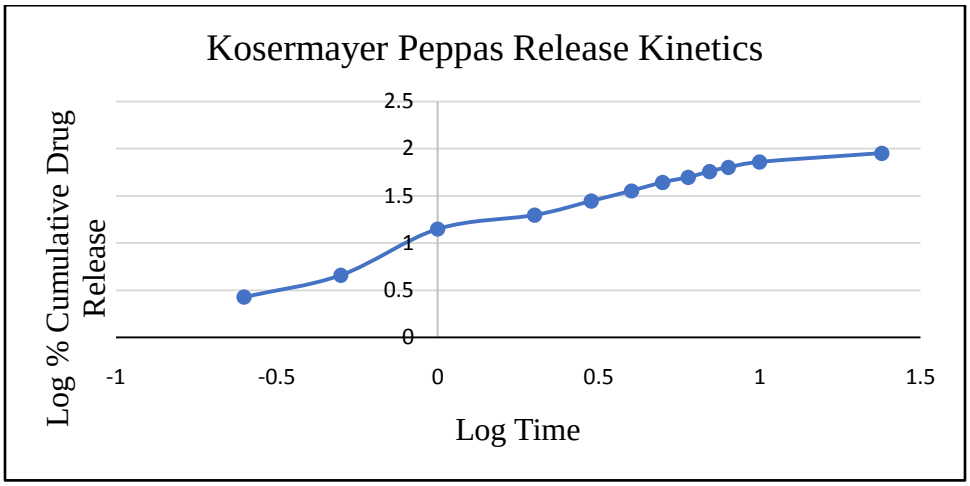

Figure 11 In vitro log % cumulative release verses log time graph (Kosermayer Peppas release model) of Pro-CBP-Gel

Table 6 Selection of release mechanisms for Pro-CBP-Gel

Model	**Equation and R^2**
Zero Order	y = 3.7709x + 17.839 R^2 = 0.7885
First order	y = -0.0422x + 1.9636 R^2 = 0.9719
Higuchi matrix system	y = 22.148x - 7.1295 R^2 = 0.954
Kosermayerpeppas	y = 0.8261x + 1.0178 R^2 = 0.9642

Pro-CBP-Gel follows first order release kinetic, with an R^2 value of 0.9719, representing drug release from proniosomal gel is concentration dependent.

3.8 Stability Study

Table 7 Stability study data for Pro-CBP-Gel

Parameters	**Room temperature**			**2-8**°C		
	1 month	**3 month**	**6 month**	**1 month**	**3 month**	**6 month**
pH	6.65	6.62	6.54	6.69	6.64	6.61
Viscosity (cps)	25000	25000	25000	25000	25000	25000
Phase separation	No	No	No	No	No	No
Spreadability (gm cm/sec)	13.69	13.90	14.21	13.67	13.72	13.62
% Entrapment efficiency	89.22%	87.19%	84.63%	93.12%	93.01%	92.55%
% Drug content	96.22%	95.21%	94.66%	98.70%	97.18%	97.10%

Particle size	0.883 µm	1.12 µm	1.13 µm	0.398 µm	0.493 µm	0.926 µm
Zeta potential	-50.1	-83.2	-79.7	-53.4	-89.9	-88.8

4 Discussion

The proniosomal gel was developed by co-acervation phase separation method. Selection of non-ionic surfactant, membrane stabilizer, solvent, drug concentration was done by simultaneous optimization method.Selection of levels of independent variables was done for further development of formulation.Software Design Expert (Version 13.0.5.0, Stat-Ease Inc, Minneapolis, MN) was used to optimize and evaluate main effects, interaction effects of the formulation. A 3^2 Design was used, the design contained two independent variables i.e. amount of surfactant and amount of phospholipon 90H which were varied at three levels i.e. high, medium and low.

Dependent variables chosen were Percentage drug entrapment efficiency and percentage cumulative drug release at 24 hr. Study design suggest that both surfactanct and lipid concentration affect the entrapment efficiency i.e. both are singinificant term where as in case of % cumulative drug release only lipid was found to be the significant factor.

All the Proniosomal batches provided by software were evaluated for in vitro release, % Entrapment efficiency, vesicle size, zeta potential, drug content. Selection of optimized batch was done from the software based on desirability.Optimized batch was then incorporated into Carbopol gel to get final Pro-CBP-Gel.

Pro-CBP-Gel was evaluated for various parameters like pH, Viscosity, Spreadability, Extrudability, Zeta potential, Vesicular size, in vitro drug release, etc.A comparison study was also done for in vitro drug release of Pro-CBP-Gel with various formulations of resveratrol i.e., Res-CBP-Gel, Res-Cream and the optimized proniosomal gel without Carbopol gel.Pro-CBP-Gel found to follow first order release kinetics.Stability study revealed that, proniosome remains more stable at 2-8°C ± 0.5°C as compared to 25°C ± 0.5°C.

5Conclusion

The present study showed that proniosomal gel is a suitable carrier for the delivery of Resveratrol with enhanced transdermal delivery. The optimization studies clearly indicated that entrapment efficiency depends both on the concentration of surfactant and lipid, and permeation depends on the concentration of lipid only. Hence, the study shows that the formulation enhances the penetration of drug through the skin, and the optimized formulation did not show any formulation problems associated with it. The gel also passes the short-term stability studies, indicating the physical and chemical stability of the product. Thus, the developed topical proniosomal formulation may prove to be a promising carrier for Resveratrol and other drugs, especially due to their simple production and simplistic scale up.

6 Acknowledgement

We sincerely thank Meyer organics for providing us with gift samples of drug and phospholipon90H and 90G. We also thank Bhupal Nobles University and Maharishi

ArivindCollege of Pharmacy for providing the infrastructure facilities to conduct the research work.

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