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Proniosome-Based Transdermal Drug Delivery of Resveratrol

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Abstract

Vesicular systems have been receiving a lot of interest as a carrier for advanced drug delivery. The present investigation aimed at the development and characterization of proniosome based transdermal delivery of resveratrol. The proniosomal structure was a liquid crystalline-compact niosomes hybrid which could be converted into niosomes upon hydration by skin itself. To optimize the formulation, various proniosomes composed of various ratios of sorbitan fatty acid esters, polysorbates, cholesterol, and lecithin were prepared by the coacervation-phase separation method. From each of the prepared proniosomes, the entrapment efficiency with span 60 was found to be the highest. The effects of cholesterol, phospholipids, and solvents were also assessed. Optimized formulation was characterized for entrapment efficiency, scanning electron microscope, transmission electron microscope, *in vitro* release study, vesicle size, and stability studies at 4°C and room temperature. Thus, proniosome was found to be a promising carrier system for resveratrol because of ease of preparation and better bioavailability.

Keywords: Permeation, Proniosomes, Resveratrol, Transdermal drug delivery

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Introduction

Encapsulation of the drug in vesicular structures is one such system, which can be expected to prolong the duration of the drug in systemic circulation and to reduce the toxicity by selective up taking. Drug delivery systems using colloidal particulate carriers such as liposomes or niosomes have proved to possess distinct advantages over conventional dosage forms because the particles can act as drug reservoirs, can carry both hydrophilic drugs by encapsulation or hydrophobic drugs by partitioning these drugs into hydrophobic domains and modification of the particle composition or surface can adjust the drug release rate and/or the affinity for the target site. Although niosomes as a carrier have shown advantages such as being cheap and chemically stable, they are associated with problems related to physical stability such as fusion, aggregation, sedimentation, and leakage on storage.[1] All methods traditionally used for the preparation of niosomes are time-consuming and many involve specialized equipment.

To overcome the disadvantages associated with niosomes, proniosomes are prepared and reconstituted into niosomes. For transdermal delivery, proniosomes are the best vesicular system because they act as a drug reservoir for a prolonged period and increase skin permeation. The formulation of drugs into proniosomes also helps in better physical and chemical stability of the drug and the vesicular nature of the delivery system helps the drug to permeate through the skin with an ease and helps in reaching systemic circulation and the target site without losing any drug activity and providing better therapeutic efficacy.

Resveratrol (trans-resveratrol; trans-3,5,4′-trihydroxy -stilbene) is a polyphenolic compound from the stilbens family, which is mainly found in grape skins, peanut, and the compound was first isolated from the roots of Polygonum cuspidatum, Fallopia japonica Family: *Polygonaceae*, a plant used in traditional Chinese and Japanese medicine.[2] Resveratrol has been reported to have various benefits for human health, including anti-aging, antioxidant, anti-inflammatory, anti-carcinogenic, anti-obesity, and heart/brain protective effects. However, the utilization of resveratrol as a nutraceutical currently limited due to its poor water solubility, high chemical instability, and low oral bioavailability.

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Resveratrol belongs to BCS class IV drug and has low bioavailability due to its rapid metabolism into glucuronate and sulfonated conjugate forms. Encapsulation of resveratrol can be used to improve its physical stability, chemical stability, protection from light, and bioavailability.[3]

Transdermal administration and encapsulation of resveratrol can improve its physical stability, chemical stability, protection from light, avoid first-pass metabolism, and thus enhances bioavailability.

Materials and Methods

Materials

Span-20, 40, 60, 80 (S.D. fine chemical limited, Mumbai), Tween-40, 60, 80 (S.D. fine chemical limited, Mumbai), Sodium Hydroxide Pellets extra pure and Potassium dihydrogen Orthophosphate anhydrous extra pure (Molychem, Mumbai), Carbopol 940 (S.D. fine chemical limited, Mumbai), Ethanol LR Grade (Himedia Laboratories Pvt. Ltd., Mumbai), Resveratrol was obtained as a gift sample from Meyer Organics Pvt. Ltd., Mumbai, Phospholipon 90H was obtained as a gift sample from Lipoid GmbH, Germany. Distilled water was used for all experiments.

Preparation of Proniosomes

Proniosomes were prepared by Conservation Phase Separation Method.[4] A precisely weighed amount of surfactant (Span 60), lecithin, cholesterol, and Drug were taken in a clean and dry amber-colored wide-mouthed glass container. To this absolute alcohol (ethanol) was added and the container was covered with a lid to prevent loss of Solvent from it. The above mixture was warmed in a water bath at 60°C–70°C until the Surfactant mixture dissolved completely. Then the Phosphate Buffer Solution PBS (pH 7.4) was added and warmed in a water bath till a clear solution was formed. The above mixture was allowed to cool down at room temperature until the dispersion was converted to Proniosomal gel (PNG).

To select the organic solvent PNG 16 formulation was chosen as it showed the highest entrapment of drug. The selected formulation was prepared with Ethanol, Propanol, Butanol, and Iso-propanol. To optimize the amount of drug which can be loaded, the selected formulation was prepared with different amounts of drug, i.e., 5 mg, 10 mg, 15 mg 20 mg, and 25 mg. 1% w/v of Carbopol 940 gel was prepared in distilled water and mixed with proniosomes to produce 1% Resveratrol PNG. The final PNG obtained was preserved in the dark for further use.[5]

Evaluation of Proniosome

Optical microscopic examination

Hydration of PNG (100 mg) was done by adding phosphate buffer solution pH 7.4 (PBS pH 7.4) (5 mL) in a small glass vial with occasional shaking for 10 min. Small amounts of the formed niosomes were spread on a glass slide and examined for the niosomal vesicles structure and the presence of insoluble drug crystals. Photomicrographs were taken for niosomes using future winjoe projection microscope (MEM 1300, Chin) using 10× magnification power.[6]

Vesicle size analysis[7]

Size and size distribution studies were done for niosomes prepared from proniosomes hydration with agitation (shaking). The analysis was done by adding double distilled water to the PNG (100 mg) in a small ambar coloured glass vial with occasional shaking for 10 min. After hydration, the dispersion of niosomes were analyzed for their size and polydispersity index on Zetasizer Nano ZS, Malvern instruments, based on photon correlation spectroscopy, at a scattering angle of 90° and temperature of 25°. Each measurement was the results of 12 run. Results were the means of triplicate experiments.

Zeta potential analysis (surface charge)[8]

Zeta potential of the optimized formulation was done after hydration with double distilled water using Zetasizer Nano ZS, Malvern instruments. The measured values were corrected to a standard reference at temperature of 20°C. Results are the means of triplicate experiments.

Scanning electron microscopy (SEM)[9]

The prepared optimized PNG was also characterized for their surface morphology (roundness, smoothness, and formation of aggregates) using SEM. Hydration of PNG was done with double distilled water. One drop of proniosomal suspension was mounted on a SEM holder with double-sided adhesive tape, IR dried, and sputter coated with a layer of platinum. The sample was then examined using a SEM (FEI QUANTA 200, gatar, Netherlands) equipped with a digital camera, at 25kV and 80000× magnification.

Transmission electron microscopy (TEM)[9]

The morphology of hydrated niosomal dispersion prepared from proniosome as also determined by TEM. A drop of niosomal dispersion was applied to a carbon-coated 300-mesh copper grid and left to adhere to the carbon substrate for about 1 min. The remaining dispersion was removed by a piece of filter paper. A drop of 1% aqueous solution of uranyl acetate was applied for 2 min and again the solution in excess was removed by the tip of filter paper. The sample was air-dried and observed under the TEM (TECNAI 20 Philips, 200kv TEM, Holland) at 200 KV.

Entrapment efficiency[10]

Entrapment efficiency was carried out by centrifugation method. The entire batch of proniosome gel was hydrated with 10 mL of pH 7.4 phosphate buffer and it was bath sonicated for about 10 min. The aqueous dispersion containing niosomes were separated from unentrapped drug by centrifugation at 15,000 rpm and at 4°C for 30 min. The supernatant was separated and the pellets were again washed with another 10 mL of pH 7.4 phosphate buffer and both supernatants were mixed and the drug in the supernatant was determined spectrophotometrically at 305 nm. All the spectrophotometric analysis was carried out in triplicate and the values were averaged. Entrapment efficiency was obtained by using the following formula.

$$\%\,Entrapment\ efficiency = \frac{\text{⌣⌣⌣⌣⌣⌣⌣}}{Total\ amount} \times 100$$

In-vitro release study[11]

The release of drug from proniosomes was determined using the dialysis tubing method. The protocol to conduct the study in shown in Table 1. The dialysis membrane was soaked in pH 7.4 phosphate for 24 h before use for activation. Proniosome gel was first hydrated with pH 7.4 phosphate and the suspension equivalent to 10 mg Resveratrol was taken in dialysis bag and the bag was then placed in a beaker containing 75 mL of 1:1 pH 7.4 phosphate: ethanol. The beaker was placed over a magnetic stirrer having a stirring speed of 100 rpm and the temperature was maintained at 37 ± 1°C. 5 mL aliquots were withdrawn periodically and were replaced by a fresh diffusion medium. The withdrawn samples were appropriately diluted and analyzed for drug content using UV spectrophotometer at 305 nm keeping the diffusion medium blank. All determinations were done in duplicate and values were averaged. A comparative *in vitro* release study was also performed by making resveratrol Carbopol gel.

Data analysis via drug release kinetics study[12]

The mechanism of resveratrol release from proniosomal formulations was determined using the following mathematical models: zero-order kinetics, first-order kinetics, Higuchi kinetics, and Korsmeyer-Peppas kinetic by plotting a graph of % cumulative

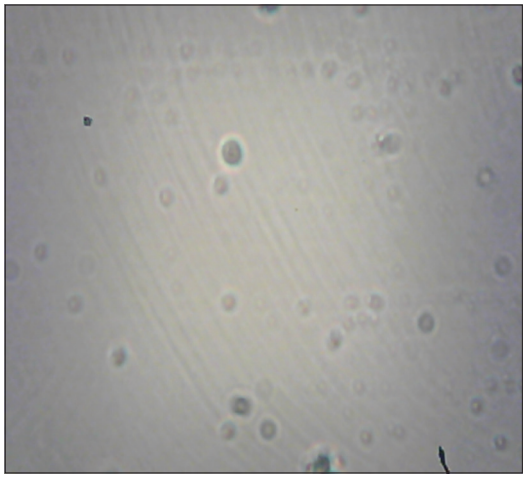

Figure 1: Photomicrographs of proniosomes

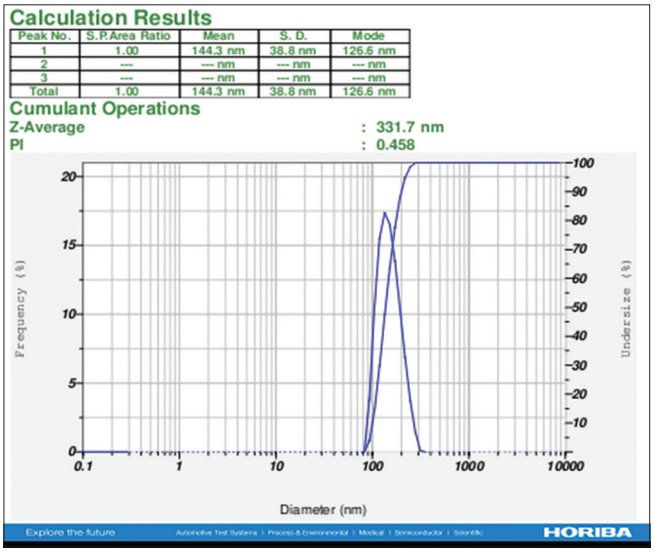

Figure 2: Vesicle size analysis graph

release against time, log % cumulative release against time, % cumulative release against the square root of time, and log % cumulative release against log time, respectively.

Drug content[13]

Weighed accurately 100 mg of optimized PNG and transferred it to 50 mL volumetric flask. Gel was then dissolved and made up the volume up to the mark with methanol. The absorbances were determined by UV visible Spectrophotometry at 305 nm using methanol as blank. Appropriately diluted if needed. The placebo interference was checked before analysis. The % drug contents were found out by calibration curve. The experiment was carried out in triplicate.

Histopathological investigation of the skin using PNG[14,15]

The rat abdominal skin region measuring approximately 4 cm^2 was mounted on a modified Franz diffusion cell. The PNG was applied identically to diffusion study and the effects were compared

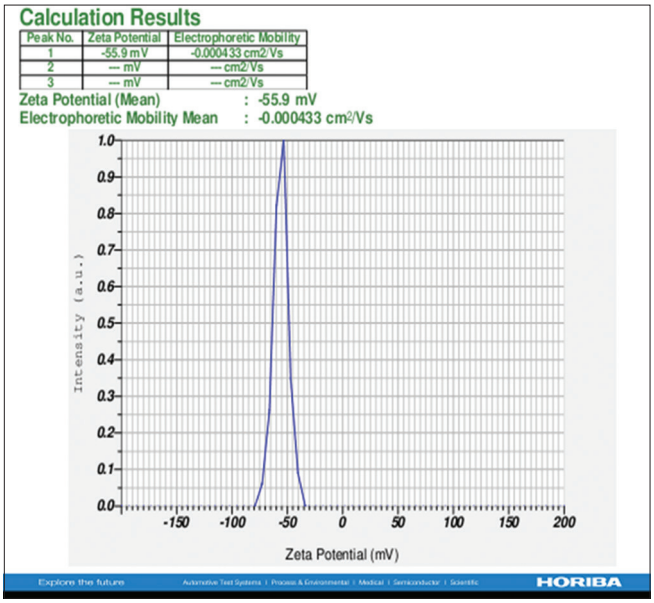

Figure 3: Zeta potential analysis graph

against phosphate buffer pH 7.4 as control. After collection the tissues were immediately preserved in the 10% neutral buffered formalin, processed by routine method for histological observation. Processed tissues were sectioned (at 5 um) and taken on the clean glass slides and stained by hematoxylin and eosin and observed under microscopes at different magnifications. Sections were examined histologically to check any microscopic alterations of pathological significance.

pH determination[16]

PH of Formulation: The pH of product can influence not only the solubility of the drug in the formulation, but may also affect its potential to cause skin irritation. The pH of optimized batch (PNG 16) was measured using pre-calibrated digital pH meter (Equip-Tronics, EQ-610, India). One gram of gel was dispersed in 25 mL of distilled water and the electrode was then dipped into gel formulation until constant reading was obtained. And constant reading was noted. The measurement of pH was replicated 2 times and the results were averaged.

Spreadability[17,18]

The spreadability of optimized batch (PNG 16) was evaluated to test the ease of applicability on the skin. It was determined by Wooden block and glass slide apparatus which was fabricated in laboratory and used for this study. 2.5 g of PNG incorporated into carbopol gel was placed between two slides. 100 g weight was then placed on upper slide for 2 min to compress the formulation to the uniform thickness and then 50 g weight was placed on pan and the time was noted for the upper slide (movable) to separate completely from the fixed slides. This experiment was carried out in triplicate and the results were averaged. Spreadability was calculated using the following formula.

$$S = \frac{M \times L}{T}$$

Where, S = Spreadability in g.cm/s

M = Weight tied to upper slide (50 g)
L = Length of lower glass slide (30 cm)
T = Time taken to separate the slides (seconds).

Viscosity determination[17]

The flow characteristic of topical formulation depends upon their viscosities. Changes in viscosity of the product are indicative of changes in the stability of the product. The viscosity of the optimized formulation was measured in triplicate using Brookefield viscometer with spindle number 25 mL beaker at room temperature. The spindle was lowered into beaker containing gel and rotated at, 100 rpm. At each speed, the corresponding dial reading on the viscometer was recorded. The following formula was used to calculate the viscosity in centipoises.

Viscosity (cps) = Spindle reading × Factor for spindle no. used

Factor for spindle no. 6 is 100 and dial reading was found to be 25.

In vitro antioxidant activity evaluation[19-21]

There are various methods available for antioxidant evaluation like, superoxide radical scavenging assay, hydroxyl radical scavenging assay, nitric oxide radical scavenging assay, anti-lipid peroxidation assay and 1,1-diphenyl-2-picryl hydrazyl (DPPH)-free radical scavenging assay. The present work involved the *in vitro* antioxidant activity study of optimized formulation (PNG 16) by DPPH free radical scavenging activity.

Preparation of standard solution (positive control)

10 mg of ascorbic acid was dissolved in 10 mL ethanol to give 1000 ppm solution of ascorbic acid. From this 1 mL was taken and further diluted to 10 mL with ethanol to gel 100 ppm solution. Further dilutions were done to get concentration of 10, 20, 40, 60, 80, and 100 ppm solution.

Preparation of test sample

Optimized PNG equivalent to 10 mg Resveratrol was dissolved in 10 mL ethanol to get 1000 ppm solution. This was considered a stock solution. Further dilutions were done to get concentration of 10, 20, 40, 60, 80, and 100 ppm solution.

Preparation of DPPH solution

The DPPH solution of strength 0.1 mM was prepared in ethanol using below-mentioned formula:

$$Molarity = \frac{weight\ in\ gram}{molecular\ weight \times volume\ in\ ml} \times 100$$

Where molecular weight of DPPH is 392.34.

Methodology

The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of 1 mL of 0.1 mM DPPH in ethanol, 1 mL of methanol, and 0.5 mL of test sample at various concentrations (10–100 ppm). Ascorbic acid was used as a positive control. The absorbance of the mixture was taken at 516 nm after 30 min of incubation period. The mixture of 1 mL of 0.1 mM DPPH in ethanol and 1 mL of methanol serves as blank (control). The percentage scavenging activity was determined by comparing the results of the test sample with standard ascorbic acid. The radical scavenging activity was expressed as the inhibition percentage and monitored as per the equation:

$$\% \text{ Inhibition} = [(A_{Control} - A_{Test}) \times 100] / A_{Control}$$

IC_{50} values were calculated.

Stability study[22]

Stability studies were carried out by storing the optimized formulation (PNG 16) at various temperature conditions, i.e., at refrigeration temperature (2°–8°C), room temperature (25° ± 0.5°C) for a period of 3 months. Throughout the study, proniosomal formulations were stored in amber-colored glass bottles. The samples were withdrawn at different time intervals over a period of 1–3 months and analyzed for drug content, vesicle size, zeta potential, and entrapment efficiency.

Results

Proniosomes were prepared by co-acervation phase separation method. Different formulations were prepared for the selection of surfactant, membrane stabilizer, lipid, and concentration of drug that can be used in optimized formulation.

Among all, span 60 was selected as representative non-ionic surfactant, because the entrapment efficiency with span 60 was found to be the highest [Table 1, PNG1 –PNG 4]. Spans follow the trend span 20 < span 40 <span 60> spans 80 for entrapment efficiency. In case of tweens, gel formation was not occurred (PNG 5-PNG 7).

Maximum entrapment efficiency was obtained when 60mg cholesterol was used as membrane stabilizer (PNG 8-PNG 10). On the further increase of cholesterol concentration competes with the drug for the space within the bilayers, hence excluding the drug resulting in decreased entrapment efficiency. Among phospholipon 90H and phospholipon 90G, phospholipon 90H showed maximum entrapment hence it was selected as membrane stabilizer and penetration enhancer (PNG 11 and PNG 12). Ethanol was selected as the organic solvent as the vesicles were formed more by this. Moreover, even texture and overall appearance of the formulation were good compared to other organic solvent like isopropyl alcohol and Butanol.

It was observed that maximum entrapment efficiency was obtained when 10 mg drug was used; further increase in drug concentration decreases entrapment efficiency. Hence, 10 mg drug was selected for further development of the design.

From Table 2 (PNG13-PNG20), it was observed that the maximum drug entrapment was obtained up to the ratio of span 60: lipid: Cholesterol at a ratio 4:4:1, respectively. It can be concluded that as the ratio of surfactant and lipid was increased with constant cholesterol there was increased in entrapment efficiency from 1:1:1 to 4:4:1, further increase in ratio leads to decrease in entrapment efficiency. The optimized formulation, i.e., PNG 16 was further evaluated.

Table 1: Protocol of *in vitro* study using dialysis tubing method

Membrane used	*Cellophane membrane (60 μm)*
Temperature (°C)	37±1
Dissolution medium	pH 7.4 phosphate buffer: Ethanol (1:1)
Total volume	75 mL
Replacement volume	5 mL

Table 2: Composition, entrapment, and appearance of different formulations

Formulation code	*Composition*	*Ratio of surfactant: Lipid: Cholesterol*	*Percentage entrapment efficiency*	*Observations*
PNG 1	Span 20:P 90H: C	1:1:1	40.89	Creamy gel
PNG 2	Span 40:P 90H: C	1:1:1	49.45	Creamy gel
PNG 3	Span 60:P 90H: C	1:1:1	62.22	White semisolid gel
PNG 4	Span 80:P 90H: C	1:1:1	53.66	Transparent gel
PNG 5	Tween 40:P 90H: C	1:1:1	_	Gel formation not occurred
PNG 6	Tween 60:P 90H: C	1:1:1	_	Gel formation not occurred
PNG 7	Tween 80:P 90H: C	1:1:1	_	Gel formation not occurred
PNG 8	Span 60:P 90H: C	1:0:1	76.22	White semisolid gel
PNG 9	Span 60:P 90H: C	1:0:2	62.54	White semisolid gel
PNG 10	Span 60:P 90H: C	2:0:1	67.88	White semisolid gel
PNG 11	Span 60:P 90H: C	1:1:1	83.22	White semisolid gel
PNG 12	Span 60:P 90G: C	1:1:1	69.91	White semisolid gel
PNG 13	Span 60:P 90H: C	1:1:1	81.22	White semisolid gel
PNG 14	Span 60:P 90H: C	2:2:1	89.90	White semisolid gel
PNG 15	Span 60:P 90H: C	3:3:1	91.37	White semisolid gel
PNG 16	Span 60:P 90H: C	4:4:1	96.15	White semisolid gel
PNG 17	Span 60:P 90H: C	5:5:1	89.07	White semisolid gel
PNG 18	Span 60:P 90H: C	6:6:1	88.57	White semisolid gel
PNG 19	Span 60:P 90H: C	7:7:1	87.66	White semisolid gel
PNG 20	Span 60:P 90H: C	8:8:1	85.58	White semisolid gel

Amount of drug used: 10 mg, 1 unit≡60 mg. PNG: Proniosomal gel, P 90H: Phospholipon 90H, *P* 90G: Phospholipon 90G

Table 3: *In vitro* release of resveratrol

Time (h)	*Percentage cumulative release*	
	Resveratrol carbopol gel	*Optimized batch*
0.25	0.06	2.67
0.5	0.67	4.56
1	1.32	14.06
2	3.67	19.76
3	5.51	27.84
4	6.91	35.72
5	8.12	43.99
6	9.71	49.68
7	11.01	57.37
8	11.92	63.39
24	21.22	88.12

Optical Microscopic Examination

Optical microscopic examination showed vesicles are very small in size as shown in Figure 1 and confirmed the formation of vesicles by the applied method.

Vesicle Size Analysis

Vesicle size was found to be 331.7 nm as observed in Figure 2. The polydispersity index was also provided automatically by the instrument and it was 0.458 which indicates broad particle size distribution. This particle size is accepted for topical use.

Zeta Potential Analysis

The zeta potential of the optimized batch was found to be −55.9 mV as shown in Figure 3, which proves that vesicles had a strong negative charge which concludes that vesicles repel each other. And hence they will not aggregate or coalesce. This was also seen in SEM and TEM that there is no aggregation of vesicles.

SEM

SEM images [Figure 4] indicated that the niosome formed after hydration was of nanometer size dimensions with smooth surface, spherical, and were homogeneously distributed.

Table 4: Release mechanisms of proniosomal resveratrol gel

Model	*Equation and R^2*
Zero order	y=3.5503x+16.947
	R^2=0.7951
First order	y=−0.0388x+1.9507
	R^2=0.9707
Higuchi matrix system	y=0.0453x+0.3818
	R^2=0.9575
Korsemeyer-peppas	y=0.8241x+1.0154
	R^2=0.9599

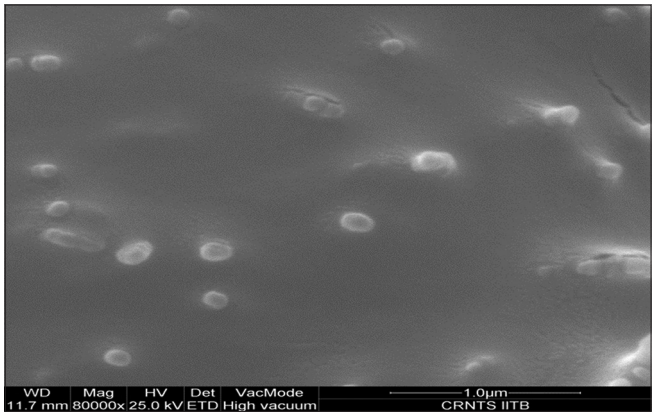

Figure 4: Scanning electron microscope image of niosomes formed after hydration of proniosomal gel

TEM

Results of transmission electron microscopic study [Figure 5] of niosomes prepared from Resveratrol PNG formulations show the vesicles are well identified, spherical, and discreet with sharp boundaries having large internal aqueous space.

Entrapment Efficiency

As shown in Table 2, all the formulation batches showed very good entrapment efficiency. Resveratrol is a hydrophobic drug

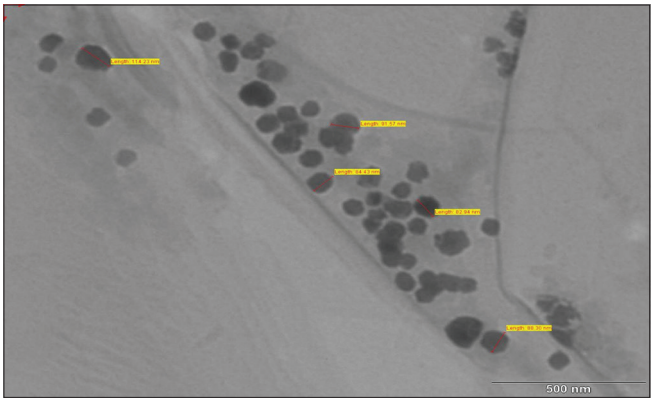

Figure 5: Transmission electron microscopy image of niosomes formed after hydration of proniosomal gel

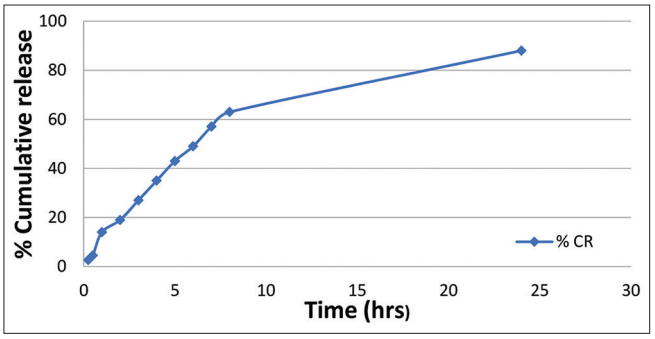

Figure 6: *In vitro* % cumulative release versus time of sample withdrawal graph (Zero order) of Resveratrol proniosomal formulations

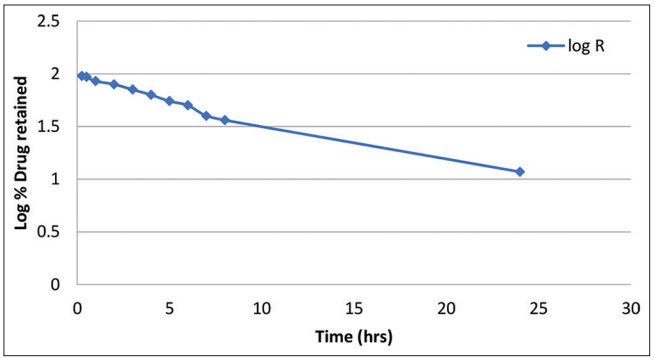

Figure 7: *In vitro* log % Drug retained versus time of sample withdrawal graph (First order) of from Resveratrol proniosomal formulations

and must be entrapped within the hydrophobic tail of noisome. Batch containing Span 60: Phospjolipon 90H: Cholesterol in the ratio 4:4:1 respectively, showed maximum entrapment efficiency because the drug leaching from vesicle is low due to high phase transition temperature and low permeability of surfactant.

In-vitro Release Study

In vitro release study of the optimized batch was performed by dialysis tubing method. As seen in the Table 3 plain gel released only 21% of the drug in 24 h but PNG released 88% of drug which is better than plain gel.

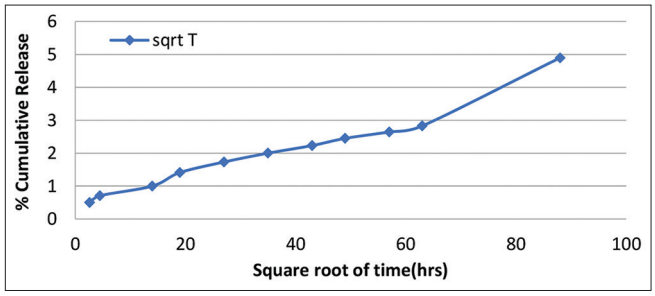

Figure 8: *In vitro* log % cumulative release versus square time of sample withdrawal graph (Higuchi release) of Resveratrol from proniosomal formulations

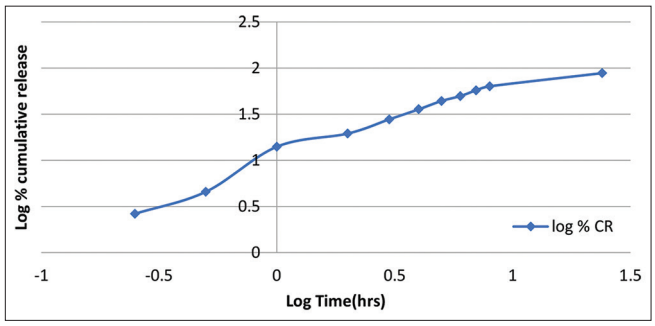

Figure 9: *In vitro* log % cumulative release versus log time of sample withdrawal graph (Korsmeyer release model) of Resveratrol from proniosomal formulations

Table 5: Proniosomal gel evaluation

Test	*Observations*
pH	6.76
Spreadability	18.5 g cm/s
Viscosity	15,000 cps

Data Analysis through Drug Release Kinetics study

By comparing the various graphs from Figures 6-9, it was found that Resveratrol PNG follows first-order release kinetic, with an R^2 value of 0.9707 as seen in Table 4, reprenting drug release from PNG is concentration dependent.

Drug Content

Good drug content of 99.45% was achieved making the formulation economical without wastage of the drug. This value is acceptable for formulation and it shows that there is no leakage degradation occurred during formulation.

Histopathological Investigation of Skin Using PNG

Control: NAD (No Lesion of pathological significance was observed)

Test: NAD.

The histology of excised rat skin in control and treated with optimized PNG after 24 h is shown in Figure 10. The microscopic observations indicate that the optimized batch has no significant effect on the microscopic structure of the skin. The surface epithelium lining and the granular cellular structure of the skin were totally intact. No major changes in the ultra-structure of

Table 6: Percentage inhibition by optimized batch at different concentration

Test sample	*Percentage inhibition at concentration (µg/mL) (%)*						*IC_{50} (µg/mL)*
	10 µg/mL	*20 µg/mL*	*40 µg/mL*	*60 µg/mL*	*80 µg/mL*	*100 µg/mL*	
Formulation (PNG 16)	12.71	40.53	63.654	78.56	80.44	86.23	58.88
Positive control (ascorbic acid)	17.41	48.09	69.56	83.12	87.46	93.78	65.10

PNG: Proniosomal gel, IC_{50}: Half-maximal inhibitory concentration

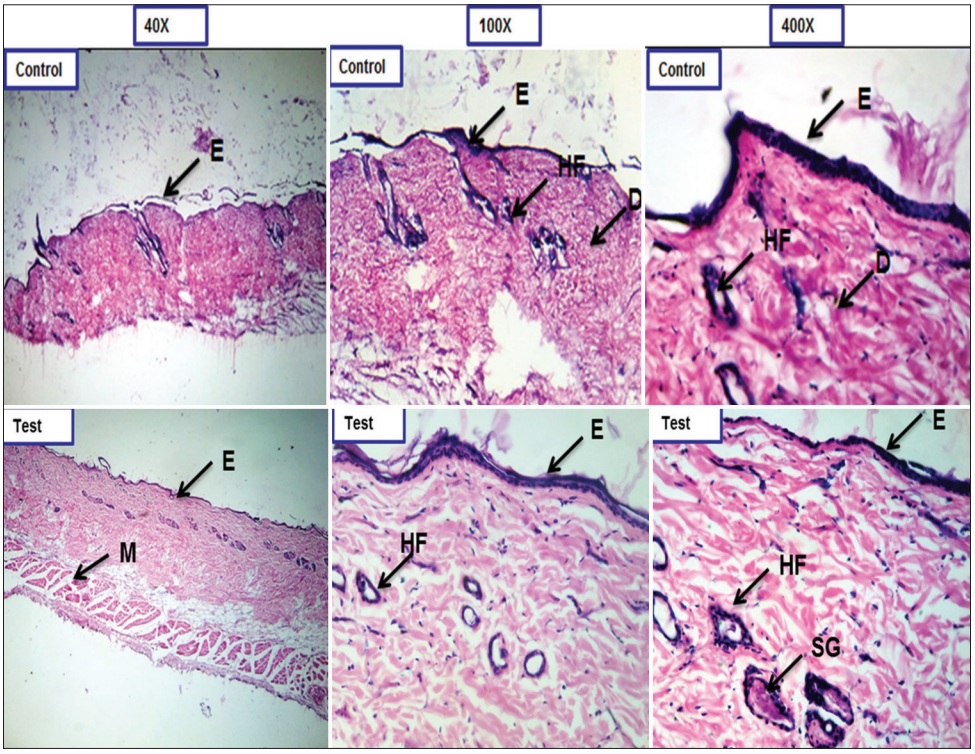

Figure 10: Histopathological investigation of skin

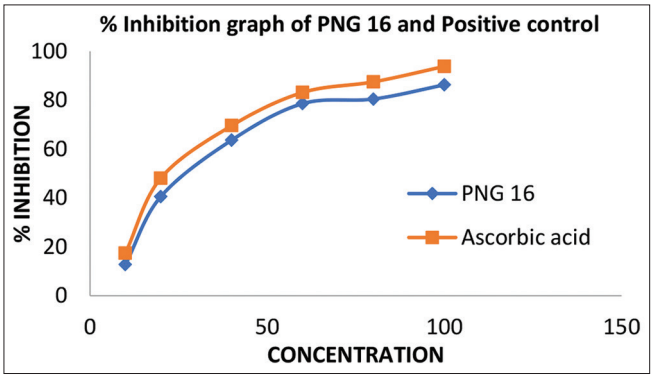

Figure 11: Graph of % inhibition against diphenyl-2-picryl hydrazyl

skin morphology could be seen and the epithelial cells appeared mostly unchanged indicating skin compatibility.

pH, Spreadability and Viscosity

The pH [Table 5] of optimized formulation was found to be 6.76 which is in range with the physiological skin surface pH indicating skin compatibility. Spreadability and viscosity of optimized PNG formulation revealed optimum spreadability and consistency respectively, due to incorporation in carbopol 940 (1%) gel matrix, which made the formulation more suitable for the topical administration.

In vitro Anti-oxidant Activity Evaluation

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidant, because of the reaction between antioxidant molecule and radical progresses, which results in the scavenging of the radical by hydrogen donation.

The percentage scavenging activity against DPPH ranged from 12.71% to 86.23% and 17.41 to 93.78% for PNG and ascorbic acid, respectively. It is seen from the above Table 6 and graph [Figure 11] that results are comparable and hence optimized PNG of Resveratrol showed strong antioxidant activity. IC_{50} value of optimized PNG and ascorbic acid was found to be 58.88 ppm and 65.10 ppm.

Table 7: Stability studies data for 3 months of the optimized batch

Parameters	*Room temperature*			*2°C–8°C*		
	1 month	*2 months*	*3 months*	*1 month*	*2 months*	*3 months*
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 (μm)		1.12	1.13	0.398	0.493	0.926
Zeta potential	−50.1	−83.2	−79.7	−53.4	−89.9	−88.8

Stability Studies

From the results of vesicle size, entrapment efficiency, drug content, and zeta potential of optimized formulation (PNG 16), it was concluded that the formulation is more stable at refrigeration temperature as compared to room temperature as shown in Table 7. It confirms that the formulation was stable at 2°C–8°C at the end of 90 days.

Conclusion

In the present study an attempt was made to develop the PNG of Resveratrol. Selection of best possible ratio of surfactant and lipid was done by simultaneous optimization method. Optimized batch was evaluated for various parameters like pH, Viscosity, Spreadability, Zeta potential, Vesicular size, SEM, TEM, *in vitro* drug release, etc. Optimized batch was evaluated for *in vitro* release and found to follow first order release kinetics.

Histopathological investigation of rat abdominal skin was done after application of optimized batch of PNG on skin. The microscopic observations indicated that the optimized batch has no significant effect on the microscopic structure of the skin.

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.

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