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Fabrication and characterization of hydroquinone in liposomal gel for transdermal drug delivery

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Abstract

This study aimed to formulate a gel for hydroquinone dermal therapy using liposomes to maintain the active agents' concentration in the skin's deepest layers. Cholesterol was incorporated to enhance the liposome's bilayer characteristics, increasing microviscosity, membrane stability, and blister rigidity. Various methods for liposome preparation exist, but the film hydration method, being the most common, was utilized here. Results for formulation HL6, which had lower levels of Lecithin Cholesterol and rotation speed, revealed a vesicle size of 180.4 nm, a Zeta potential of -37.5 mV, and an entrapment efficiency of 69.10±1.52%. In-vitro drug release data for formulations F1, F2, and F3 within 30 minutes showed hydroquinone release rates of 97.75±0.28%, 98.92±0.56%, and 94.45±0.36%, respectively. The order of drug release was F2 > F1 > F3, with F2 demonstrating the maximum release rate. The study concludes that liposomal gel is an effective transdermal drug delivery system for therapeutic molecules. Lipid vesicles, such as liposomes, are among the best mechanisms for delivering medications to their intended locations while minimizing their dissemination to non-target tissues. This liposomal gel-based formulation shows significant potential for effectively treating acne by maintaining high concentrations of active agents in the skin's deepest layers and ensuring a controlled and sustained drug release.

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INTRODUCTION

Prescription pharmaceuticals are not included in over-the-counter (OTC) medication options. It relieves pain, itches, fever, and headaches. Self-medication is the practice of taking medications on one's initiative or at the advice of a friend, relative, or other individual without first seeing a licensed healthcare provider [1]. Self-medication can assist in treating minor illnesses for which a doctor's consultation is not necessary, alleviating

Table 1 Hausner ratio, Carr's index, and angle of repose

Angle of Repose	Carr's Index	Hausner Ratio	Type of Flow
< 20°	5– 5%	-	Excellent
20–30°	12– 6%	< 1.25	Good
30–40°	18–21%	-	Fair to passable
-	23– 5%	> 1.25	Poor
-	33–38%	1.25–1.5	Very poor
-	> 40%	-	Extremely poor

Table 2 Composition of liposome

Batch No.	Lecithin (mg)	Cholesterol (mg)	Rotation Speed (rpm)
HL1	100	20	200
HL2	100	50	200
HL3	200	20	100
HL4	200	20	200
HL5	200	50	200
HL6	100	20	100
HL7	100	50	100
HL8	200	50	100

the burden on medical services, especially in developing nations with little resources for healthcare.

Drug Excipients compatibility study: A differential scanning calorimeter was used to record the thermograms. Aluminum pans with a flat bottom were filled with weighed samples (5–10 mg) and sealed tightly. These samples were heated, with alumina serving as the reference standard, between 50 and 400 degrees Celsius in a nitrogen atmosphere at a steady pace of 100 degrees Celsius per minute [8].

Formulation development

Preparation of Liposome

Lipid film hydration was used to prepare liposomes using a modified rotating vacuum evaporator. Vesicle size and drug entrapment efficiency were investigated, and the drug SPC: CHOL ratio was changed. In a nutshell, chloroform is a mixture of varied drug ratios and methanol (2:1). SPC: Using a CHOL evaporator in a vacuum at 4000.50C to create a lipid film on a flask with a circular bottom [9]. Next, the resultant lipid film was hydrated at 370±0.50C for two hours using PBS (pH 7.4). The preparation was subjected to three cycles of 30 seconds each at 40C, with a 2-minute interval between each cycle, using a probe Sonicator [Table 2]. To create liposomes, a high-pressure homogenizer was used to homogenize the mixture.

Vesicle size and entrapment efficiency were investigated thoroughly to select the significant formulation [10].

Evaluation of liposome

Vesicle size determination: The particle size analyzer (Malvern Master Sizer, Malvern Instruments Ltd., Malvern, UK) was used to measure the size of the vesicles [11].

Entrapment efficiency: The amount of Hydroquinone in liposomes was estimated using the ultracentrifugation method. The liposomal suspension was transferred to a 10 ml centrifuge tube. After diluting the suspension with 5 ml of distilled water, it was centrifuged for 20 minutes at 2000 rpm [12]. We can separate the undissolved medications in the formulation in this way. After the resultant supernatant was obtained, an appropriate volume of the protamine solution was added and held for 10 minutes. Using a calibration curve approach and ultraviolet-visible spectroscopy, the drug contents of the entrapped and entrapped drug were assessed [13].

Transmission Electron Microscopy: With TEM, surface morphology was assessed by placing a sample drop on a carbon-coated copper grid. The sample was then negatively stained with a 1% aqueous solution of phosphotungustic acid after

15 minutes. Samples were examined with transmission electron microscopy after the grid was given enough time to air dry [14]. HL6 was chosen as the ideal formulation in light of the study's findings.

Table 3 Composition of optimized liposome formulation

Formulation	Lecithin (mg)	Cholesterol (mg)	Speed (rpm)
HL6	100	20	100

Preparation of Gels

Preparation of Carbopol gel base: To create a 0.5% gel, 0.5 g of carbopol 934 was weighed, distributed in 100 ml of distilled water, and gently stirred. This was done for 24 hours [Table 4]. To keep the gel consistent, 2 milliliters of glycerin were added later. Two types of preservatives were added to the gel: methyl and propylparaben. In the same way, Carbopol gels at 1% and 2% were made [15].

Table 4 Composition of different gel base

Formulation	Carbopol (%)
F1	0.5
F2	1.0
F3	2.0

Preparation of liposomal gels: To extract the not entrapped drug, 10 milligrams of liposome formulation were dissolved in 10 milliliters of ethanol and centrifuged for 20 minutes at 6000 rpm. The supernatant was decanted after the sediment was added to the gel vehicle. Using a slow mechanical stirrer (REMI type BS stirrer, Vasai, India) at 25 rpm for 10 minutes, the hydroquinone-loaded liposomes (equal to 0.1) were incorporated into the gel. The optimized formulation used three distinct gel concentrations (0.5, 1, and 2% w/w) [16].

EVALUATION OF GEL

Determination of pH: Using a digital pH meter, the pH of each gel formulation (50 gm) was measured after being transferred to 10 ml of the beaker. For the topical gel formulation to treat skin infections, the pH should be between 3 and 9 [17].

Spreadability: The spreadability was calculated using the formula S=ml/t, where m stands for the weight of the pan attached to the higher slide, t for the distance traveled, and l for the time it took to go that distance. For practical reasons, the mass and length were determined while remaining constant. The spreadability of each formulation was assessed three times, and the average results are shown [18].

Measurement of viscosity: The viscosity of the gels was measured with a Brookfield viscometer (DV-II model). Accurate viscosity measurements were obtained using a helipath stand in conjunction with a T-Bar spindle [19].

***In-vitro* diffusion study:** An in-vitro drug release research was conducted using a modified Franz diffusion cell. The dialysis membrane (Hi Media, Molecular weight 5000 Daltons) was positioned between the donor and receptor sections. PBS pH 7.4 (24 ml) was added to the receptor compartment, and liposomal gel corresponding to 1 mg of the medication was placed in the donor compartment. Throughout the experiment, stirring occurred at 50 rpm, and the diffusion cells were kept at 37±0.5°C. A side tube was used to remove 5 ml of aliquots from the receiver compartment at various times, and a UV-visible spectrophotometer was used to determine the amount of medication present [20].

RESULTS AND DISCUSSION:

Preformulation Studies

Fourier-Transform Infra-Red Spectroscopy (FTIR): The drug's spectra were verified using FTIR spectroscopy. The distinctive peaks that resulted from particular structural traits of the drug molecule were notable. The drug's FTIR scan is displayed in Figure 1, and Table 5 lists the wave numbers.

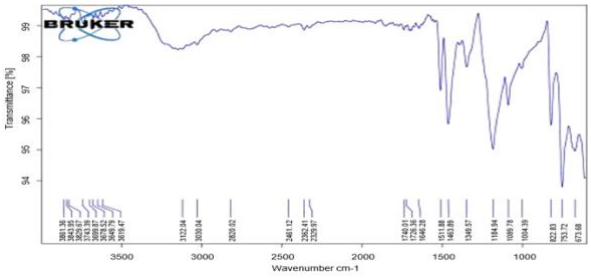

Figure 1 FTIR of Drug

Table 5 FTIR of Drug

Wave number(cm^{-1})	Inference
3829.67	O-H Str.
3122.04	=CH Str.(Aromatic)
1511.88	C=C Aromatic

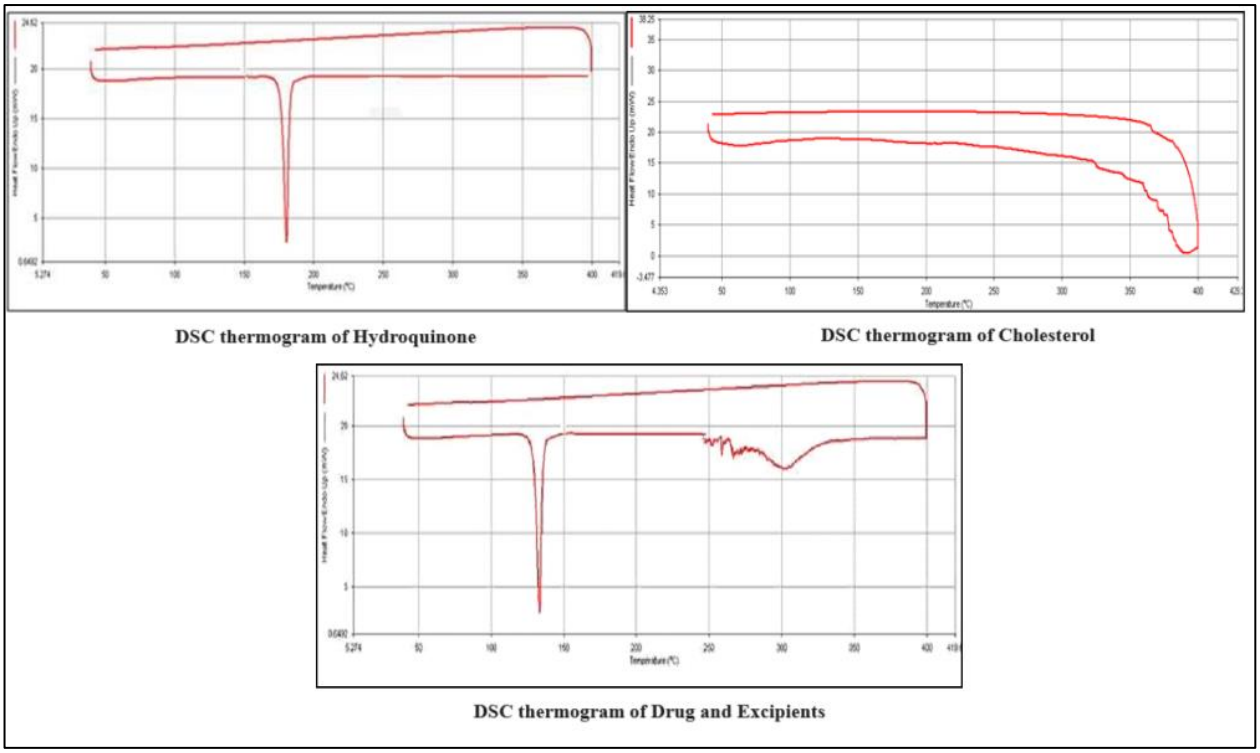

Figure 2 DSC Thermograms

Table 6 Evaluations of Liposomal Formulations

Formulation	Vesicle size (nm)	Zeta Potential (mV)	Entrapment Efficiency ((%)	Polydispersity Index (PDI ± SD)
HL1	165.3	-32.1	56.73±0.73	0.411
HL2	256.7	25.9	55.43±1.48	0.229
HL3	478.3	26.5	60.11±0.82	0.321
HL4	405.1	18.7	62.52±2.21	0.232
HL5	552.8	-32.8	64.87±1.54	0.301
HL6	180.4	-37.5	69.10±1.52	0.221
HL7	319.3	29.2	66.27±2.00	0.839
HL8	800.2	30.4	65.79±1.12	0.628

Discussion: The peak obtained indicates characteristic groups and the bonds present in the compound. Hydroquinone shows the characteristic peak at 3829.67 cm^{-1} due to OH stretching, 3122 cm^{-1} due to CH stretching vibration, and a peak at 1511.88 cm C=C stretching vibration.

Drug-Excipients compatibility study: According to DSC, the thermo gram of hydroquinone melting point is 172^{0}C, respectively. The mixture of drug and cholesterol was kept in an accelerated condition of 40^{0}C/ 75% RH for 30 days and subjected to DSC analysis [Figure 2]. The characteristic melting point of Hydroquinone did not deviate from 172^{0}C. Also, after many vigorous conditions, the DSC thermogram for the drug mixture with the cholesterol shows both individual and separate peaks, indicating that the drug is presented in its pure form and that there are no interactions between them. Also, no interaction exists between drugs and cholesterol.

Evaluation of formulations

Results observed for the vesicle size, Zeta potential entrapment efficiency, and PDI are recorded in Table 6. The vesicle size of all Liposomal formulations prepared varied between 100 and 800 nm, Zeta potential between -37.5 to 18.7mV, and entrapment efficiency between 55.43±1.48-69.10 ± 1.52 %.

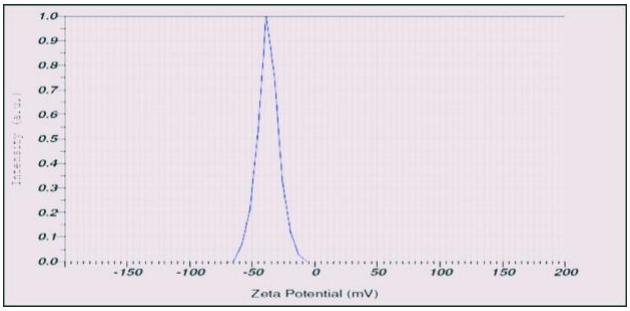

Figure 3 Results of Zeta Potential

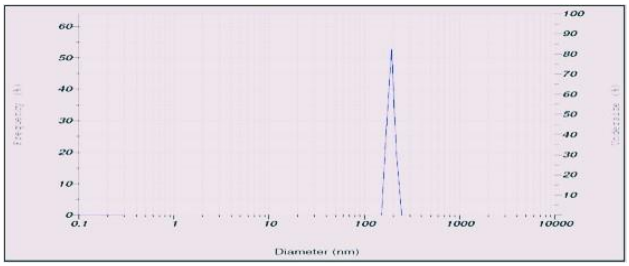

Figure 4 Results of vesicle size

Discussion: Based on the calculation of the zeta potential, entrapment efficiency, size, and polydispersity index, the formulation HL6, which has lower levels of lecithin, cholesterol, and rotation speed, was chosen as the optimal formulation. 180.4 nm was determined to be the vesicle size. Whereas Zeta potential was -37.5 mv, and entrapment efficiency was found to be 69.10±1.52%. A lower size of the liposomes is needed since they are to be entrapped into a gel. The smaller size would also be beneficial for localizing the active constituents into the deeper layer of the skin, mainly targeting the causative agent for acne. The high zeta potential indicates the stability of the formulation and the liposomal vesicles. The entrapment efficiency of the HL6 formulation was higher than that of the other formulations. The polydispersity index was lower than that of the different formulations. The index indicates the uniform size, which was lower in the case of the HL6 formulation, which suggests that the cysts present in the HL6 formulation are of uniform size, indicating a suitable formulation. Hence, based on results obtained from the study and the above discussion, HL6 was selected for Transmission electron microscopy and as an optimized formulation.

Transmission Electron Microscopy: A photomicrograph was acquired at an appropriate magnification, and transmission electron microscopy was conducted using a Hitachi H-7500 electron microscope. The photomicrograph in the Figure. The TEM characterization shows that liposomes are tiny, spherical vesicles. However, there was some variance in the size distribution, which may be explained by an uncontrollably charged neutralization process between chains with opposing charges during the liposome formation. TEM analysis showed that liposomes had a mean size of 180 nm and ranged from 100 to 200 nm [Figure 5].

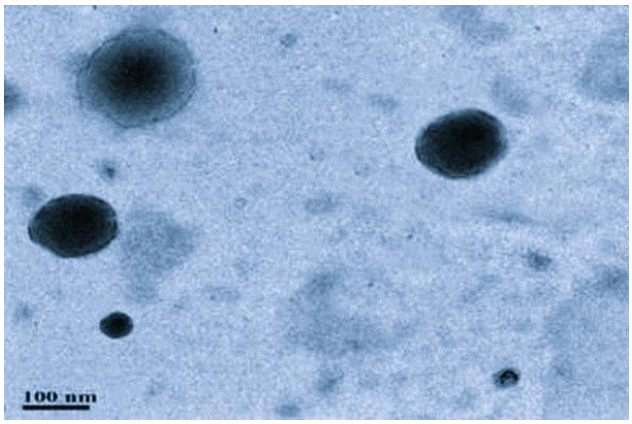

Figure 5 TEM image of liposomes

EVALUATION OF LIPOSOMAL GEL

Table 7 Assay of Hydroquinone in gel formulation

Code	Drug content
F1	99.25±2.24
F2	98.15±1.28
F3	98.75±2.46

The statistics are satisfactory, and the drug content is the most crucial aspect of liposomal formulation. That was found to be between 98.15±1.28 and 99.25±2.24 for Hydroquinone, which shows the excellent formulation capacity to hold the drug [Table 7].

pH: pH is a critical factor in transdermal drug delivery systems; the liposomal formulation results of the pH research indicate that all formulations are appropriate for transdermal delivery [Table 8]. The pH values of the prepared liposomal gels were within acceptable limits of 7.0-7.2.

Spreadability: Spreadability was measured using modified equipment. Based on the slip and drag properties of the gels, the spreadability was

calculated, and it ranged from 10.35 to 11.92 gms. cm. /sec. It is important for the gels to have optimal spreadability since high and shallow spreadability values suggest that the gel application is challenging [Table 8].

Viscosity measurements: A Brookfield viscometer was used to measure the viscosity of the gels. The rheological properties of the gels were regulated in the working area using various factors such as temperature, pressure, sample size, and so on. Table 8 shows that other constants included sample size, pressure, temperature, etc.

Discussion: The gels' viscosity also rises as the polymer concentration rises. The increased bonds between the polymer molecules, which result in a complex and dense compact mass, are the source of the viscosity increase with polymer concentration. This hardness is because gels with high polymer concentrations have less liquid than gels with low polymer concentrations. Put otherwise, and a given shear rate requires a more significant amount of shear stress the higher the polymer concentration.

Table 8 Results of liposomal gel formulations

Code	pH	Spreadability (gm. cm/sec.)	Viscosity (cps)
F1	7.2±0.022	10.35±0.045	1860±22
F2	7.0±0.035	12.02±0.022	1897±34
F3	7.1±0.029	11.45±0.029	1872±19

Discussion: According to the viscosity and spreadability values, the formulations effectively support the liposomal formulation. Additionally, the optimal viscosity of the F2 formulation allows for even application to the skin and prolonged skin contact, resulting in the greatest possible therapeutic benefit. It is also mentioned that the formulas have a pH between 7.0 and 7.2, which means they are safe to apply to the skin and won't cause irritation or other negative consequences.

***In-vitro* drug release study:** A modified Franz diffusion cell with a dialysis membrane was used to conduct an in-vitro diffusion analysis of the liposomal gel (F1, F2, and F3) for 12 hours in phosphate buffer pH 6.8. Table and Figure provide a summary of the diffusion study data. The gel release rate from liposome formulation over dialysis membrane is typically much higher than the gel's transit through the skin, demonstrating the drug-barrier qualities of the skin. During 12 hours, the drug was released from the liposomal gel in a regulated manner. It was discovered that the medication released from liposomal gel went as follows: F2>F1>F3.

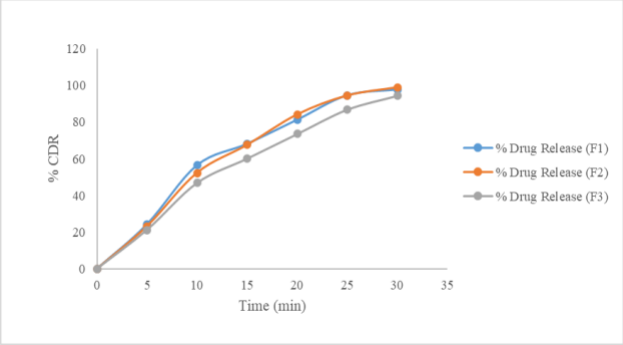

Figure 6 *In-vitro* drug release data for optimized formulation

Discussion

In-vitro drug release data for F1, F2, and F3 up to 30 minutes for Hydroquinone was found to be 97.75±0.28, 98.92±0.56 and 94.45±0.36, respectively. The release of the drug from liposomal gel was found to follow the order F2> F1> F3. The drug release study demonstrated the maximum drug release rate in the F2 liposomal gel formulation case.

CONCLUSION

In conclusion, it is possible to suggest that liposomal gel has shown to be a successful

Table 9 *In-vitro* drug release data for optimized formulation

Time (min)	Cumulative* % Drug Release (F1)	Cumulative* % Drug Release (F2)	Cumulative* % Drug Release (F3)
5	24.25±0.15	23.25±0.45	21.25±0.15
10	56.45±0.45	52.35±0.21	46.89±0.25
15	68.12±0.25	67.54±0.14	60.15±0.21
20	81.26±0.21	84.12±0.15	73.58±0.14
25	94.56±0.25	94.45±0.21	86.89±0.25
30	97.75±0.28	98.92±0.56	94.45±0.36

*Average of three readings

transdermal drug delivery vehicle for therapeutic molecules. Lipid vesicles, or liposomes, are among the best drug delivery methods for delivering a medication to its intended location while minimizing drug distribution to non-target tissues. By enabling drugs to make close touch with nearby surfaces, liposomes can improve medication absorption. Effective acne treatment may be facilitated by the liposomal gel-based formulation that was created.

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