Formulation Development and Evaluation of Topical Liposomal Gel for Management of Acne

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Abstract

The aim of the present study was to statistically optimize the vesicular formulations (Liposomes) for enhanced skin delivery of a model drug tazarotene in combination with gel contains hydroquinone. Regular 2^3 factorial designs were employed for screening of significant formulation and process variables involved in the development of liposome. The optimization batches (TL1 toTL8) were prepared by lipid film hydration method with different concentration of lecithin and cholestrol with different varying stirring speed (100 and 200 rpm) All the prepared formulation were characterized for vesicle morphology, particle size and entrapment efficiency by transmission electron microscopy (TEM). The optimized batch of tazarotene liposome TL6 was further incorporated into gel containing hydroquinone. Three different formulation (LF1, LF2, LF3) were prepared using different composition of carbopol (0.5, 1.0 and 2%) The optimized batch of tazarotene and hydroquinone incorporated gel (1%) was characterized for pH, spreadability, viscosity (cps) and *in vitro* drug release**.** The percentage drug entrapment efficiency found higher in formulation TL6, Vesicular size and drug entrapment efficiency of the optimized liposomes were found to be 180.4 nm and 69.10% respectively. *In-vitro* diffusion study demonstrated that the drug diffused from liposomal gel and conventional marketed gel was found to be 98.12% and 98.58% respectively. It can be concluded from the experimental results that the liposomal gel containing tazarotene in combination with hydroquinone has potential application in topical delivery.

Keywords: Tazarotene, Hydroquinone, Liposome, Gel, Topical Drug Delivery

INTRODUCTION

Liposome technology is one of the fastest growing scientific fields contributing to areas such as drug delivery, cosmetics, nanotechnology etc. This is due to several advantageous characteristics of liposomes such as ability to incorporate not only water soluble but also lipid soluble agents, specific targeting to the required site in the body and versatility in terms of fluidity, size, charge and number of lamellae[1]. The first report indicating that topical application of liposomally encapsulated drugs altered drug deposition was presented at the FIP 1979 congress. Mezei and Gulasekharam initiated research for utilizing liposomes as drug carriers for topical delivery in the early 1980s. With continuous efforts researchers succeeded in commercializing the liposomal gel for topical delivery of econazole in 1988 by Pevaryl T. M, Cilag, Switzerland. Mezei and Gulasekharam reported that topical application of liposomal triamcinolone acetonide for 5 days resulted in a drug concentration in the epidermis and dermis four times higher than that obtained using a control ointment, while urinary excretion of the drug was diminished. Therefore, their results indicated that the use of liposomes might be useful for increased local activity while diminishing the percutaneous absorption of the drug[2].

Depending upon their solubility and partitioning characteristics, the drug molecules are located differently in the liposomal environment and exhibit different entrapment and release properties. Highly hydrophilic drugs with log P ≤ 0.3, are located exclusively in the aqueous compartments of the liposomes. Highly lipophilic drugs, with log Poct ≥ 5, are entrapped almost completely in the lipid bilayer of the liposomes, drugs with intermediate partition coefficients, i.e., 1.7 ≤ log Poct ≤ 4, pose a major problem because they partition easily between the lipid and aqueous phases and are very easily lost from the liposomes. Such molecules form stable liposomal systems only when they form complexes with the membrane lipids[3]. However, the most problematic candidates for liposomal entrapment are the drug molecules which have poor biphasic solubility (e.g. mercaptopurine, azathioprine and allopurinol).

Liposomes are thought to act as "drug localizers" - not only as "drug transporters" [4]. This is the reason why this study focuses on liposomes as a promising form for topical drug delivery. Although most topical liposome studies have focused on drug deposition into the stratum corneum, a growing number of studies have yielded evidence of follicular delivery via liposomes[5]. Therefore the present study was aimed to statistically optimize the vesicular formulations (Liposomes) for enhanced skin delivery of a model drug tazarotene in combination with gel contains hydroquinone.

Materials and methods

Tazarotene and hydroquinone was gift sample from Bioplus life science, Banglore, India. Soya lecithin was purchased from Hi Media Ltd. Mumbai. Cholesterol was purchased from Thomas baker, methanol, chloroform, propylene glycol, was purchased from S. D. Fine Chem. Ltd., Mumbai. Ethanol was procured from Qualigens fine chemicals, Mumbai. All other chemicals were of analytical grade and double distilled water used throughout the experiment.

Optimization of formulation

Regular 2^3 factorial designs were employed for screening of significant formulation and process variables involved in the development of Liposome. Table 1 presents the description of high and low levels of various variables screened for their influence in the development of liposome of tazarotene. Table 1 depicts the L8 array for the three factors, two levels design adopted in the current studies. When the data of table 1 put into the DoE, it will provide combinations of 8 formulations as listed in table 1 as TL1 to TL8. Particle size and entrapment efficiency were the key response variables investigated thoroughly for selecting the significant formulation and response variables[6].

Method of preparation for Liposomes

Liposomes were prepared by lipid film hydration method using rotary vaccum evaporator. Drug (Tazarotene): SPC: Cholestrol ratio was altered and vesicle size and drug entrapment efficiency were studied. Briefly, a chloroform: methanol (1:2) mixture of different ration of drug (Tazarotene): SPC: Cholestrol evaporator under vaccum at 40 ± 0.5^0 C to form a lipid film on the wall of a round bottom flask. The resulting lipid film was then hydrated with phosphate buffer(pH7.4) for 2 hours at 37± 0.5 ^{0}C. The preparation was sonicated at 4 ^{0}C in 3 cycles of the 5 minutes and rest of 5 minutes between each cycle by using probe sonicator. The formulation was homogenized at 15,000 psi pressure in 3 cycles using high-pressure homogenizer to get liposome[7].

Evaluation of liposome

Vesicle size determination

Vesicle size were determined using the particle size analyzer (Horiba nanoparticle analyzer, SZ-100-S, Kyoto, Japan)

Entrapment efficiency

Tazarotene was estimated in liposome by ultra centrifugation method liposomal suspension was transferred to 10 ml centrifuge tube. This suspension was diluted with distilled water up to 5 ml and centrifuged at 2000 rpm for 20 minutes. By this we can separate undissolved drug in the formulation. Suitable volume of the protamine solution was added to the resulting supernatant and retained for 10 minutes. Liposomes were aggregated in presence of protamine and then separated

by ultra centrifugation at 15,000 rpm for 20 minutes. Supernatant and sediment were separated out. Volumes of the supernatant and sediment were measured. Sediment was diluted with distilled water up to 5 ml. The unentraped and entrapped drug contents were analyzed by estimating drug in supernatant and liposomes (sediment) by spectroscopic method[8].

Transmission electron microscopy

Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 minutes it was negatively stained with 1% aqueous solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan) [9].

Preparation of gels

Preparation of carbopol gel base

0.5 g Carbopol 934 was weighed and dispersed in distilled water with mild stirring and allowed to swell for 24 hours to obtain 0.5% gel. Later 2 ml of glycerin was added to for gel consistency.Preservatives also added to it. Similarly 1 and 2% carbopol gels were prepared [10, 11].

Preparation of liposomal gels

1g of liposome formulation was dissolved in 10ml of ethanol and centrifuged at 6000 rpm for 20 minutes to remove the unentrapped drug. The supernatant was decanted and sediment was incorporated into the gel vehicle[12].

The incorporation of the tazarotene loaded liposomes (equivalent to 0.1%) and direct incorporation of hydroquinone (4%) into gels was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer) for 10 minutes. The optimized formulation was incorporated into three different gel concentration 0.5, 1 and 2% w/w.

Evaluation of gels

Determination of pH

Weighed 50 gm of each gel formulation were transferred in 10 ml of beaker and measured it by using the digital pH meter. pH of the topical gel formulation should be between 3–9 to treat the skin infections[13].

Spreadability

A modified apparatus suggested was used for determining spreadability. The spreadability was measured on the basis of slip and drag characteristics of the gels. The modified apparatus was fabricated and consisted of two glass slides, the upper one was fixed to a wooden plate and the lower one was attached by a hook to a balance. The spreadability was determined by using the formula: S=ml/t, where S, is spread ability, m is weight in the pan tied to lower slide and t is the time taken to travel a specific distance and l is the distance traveled. For the practical purpose the mass, length was kept constant and‘t’ was determined. The measurement of spreadability of each formulation was in triplicate and the average values are presented.

Measurement of viscosity

The viscosity of gels was determined by using a Brook Field viscometer DV-II model. A T-Bar spindle in combination with a helipath stand was used to measure the viscosity and have accurate readings.

Selection of spindle

The goal was to obtain a viscometer dial or display (% torque) reading between 10 and 100, the relative error of measurement improves as the reading approaches 100. Spindle T 95 was used for the measurement of viscosity of all the gels.

Spindle immersion

The T –bar spindle (T95) was lowered perpendicularly in the centre taking care that spindle does not touch bottom of the jar.

Measurement of viscosity

The T-bar spindle (T95) was used for determining the viscosity of the gels. The factors like temperature, pressure and sample size etc. which affect the viscosity were maintained during the process. The helipath T- bar spindle was moved up and down giving viscosities at number of points along the path. The torque reading was always greater

than 10%. Five readings taken over a period of 60 sec. were averaged to obtain the viscosity.

***In-vitro* diffusion study**

An *in-vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane (Hi Media, Molecular weight 5000 Daltons) was placed between receptor and donor compartments. liposomal gel equivalent to 1 mg of Tazarotene was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 6.8 (24 ml). The diffusion cells were maintained at 37±0.5 °C with stirring at 50 rpm throughout the experiment. At different time interval, 5 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed for drug content by UV Visible spectrophotometer [14, 15].

Stability studies

Optimized formulations of tazarotene and hydroquinone loaded liposomes gel was subjected to accelerated stability testing under storage condition at 4 ± 1°C and at room temperature 25 ± 1°C. Both formulations were stored in screw capped, amber colored small glass bottles at 4 ± 1°C and 25 ± 1°C. Analysis of the samples were characterized for vesicle size and drug content after a period of 7, 14, 21 and 28 days.

Effect of storage temperature on vesicle size

Subsequent change in vesicle size of the formulations stored at 4±1°C and 25 ± 1°C was determined using a Zetasizer (Malvern Instrument, UK) after a period of 7, 14, 21 and 28 days.

Effect of storage temperature on drug content

After storage for a specified period of time of 7, 14, 21 and 28 days, the drug content of both the formulations was determined. Drug content in liposomes gel was determined spectrophotometrically to indirectly estimate the amount of drug entrapped in liposomes gel.

Results and discussion

Preparation of liposomes was based on Taguchi design and was found to be well suited and sound approach to obtain stable liposome formulation for anti-bacterial drugs. Variables such as amount of phospholipid, amount of stabilizer have a profound effect on the vesicle size and entrapment efficiency.

Drug encapsulated liposome was prepared by hot method technique and lipid film hydration technique. Vesicular carriers were characterized for drug entrapment efficiency, vesicle size, and *in-vitro* diffusion study. Vesicular size and drug entrapment efficiency of the optimized liposomes were found to be 180.4 nm and 69.10% respectively. *In-vitro* diffusion study demonstrated that the drug diffused from liposomal gel and conventional marketed gel was found to be 98.12%, in 12 hrs.98.58% in 30 min. respectively.

Cholesterol may be included to improve bilayer characteristics of liposomes, increasing the microviscosity of the bilayers which in turn reduces the permeability of the membrane to water soluble molecules. This also results in the stabilization of the membrane and increase in the rigidity of the vesicles.

In the penetration enhancing mechanism, after application of vesicles, changes in the ultra structures of the intercellular lipids were seen suggesting a penetration enhancing effect. In vesicle adsorption to and/or fusion with the stratum corneum the vesicles may adsorb to the stratum corneum surface with subsequent transfer of drug directly from vesicles to skin or vesicles may fuse and mix with the stratum corneum lipid matrix, increasing drug partioning into the skin. Liposomes have been reported to invade the skin intact and go deep enough to be absorbed by the systemic circulation.

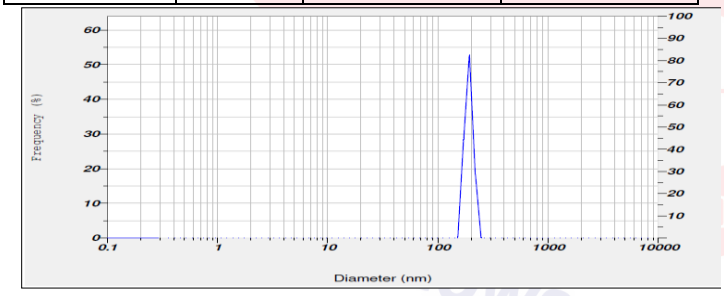
Stability studies performed for optimized liposomal gel formulations indicate that prepared gel formulations have more stability at freezing temperature than at room temperature. Therefore product should be stored at low temperatures (2-8 ^{0}C). The prepared liposomal gel formulations exhibit superior stability thereby increasing its potential application in transdermal delivery systems.

Table: 1 Composition of liposome on the basis of regular 2^3 design

Run	B. No	Lecithin (mg)	Cholestrol (mg)	Stirring Speed (rpm)
1	TL1	100	20	200
2	TL2	100	50	200
3	TL3	200	20	100
4	TL4	200	20	200
5	TL5	200	50	200
6	**TL6**	**100**	**20**	**100**
7	TL7	100	50	100
8	TL8	200	50	100

Table: 2 Evaluations of liposomal formulations of regular 2^3 designs

Formulation	Vesicle size (nm)	Zeta Potential (mv)	Entrapment efficiency (%)
TL1	165.3	-32.1	56.73±0.73
TL2	256.7	25.9	55.43±1.48
TL3	478.3	26.5	60.11±0.82
TL4	405.1	18.7	62.52±2.21
TL5	552.8	-32.8	64.87±1.54
TL6	**180.4**	**-37.5**	69.10±1.52
TL7	319.3	29.2	66.27±2.00
L8	800.2	30.4	65.79±1.12



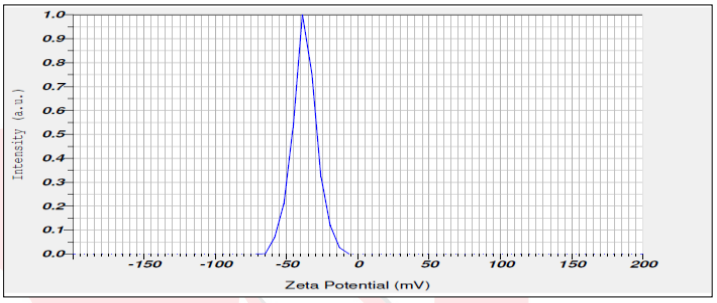


Fig. 1: Results of zeta potential

Fig. 2: Results of particle size

Table 3: Composition of optimized formulation of liposomes

Formulation Code	Lecithin (mg)	Cholesterol (mg)	Steering Speed (rpm)
TL6	100	20	100

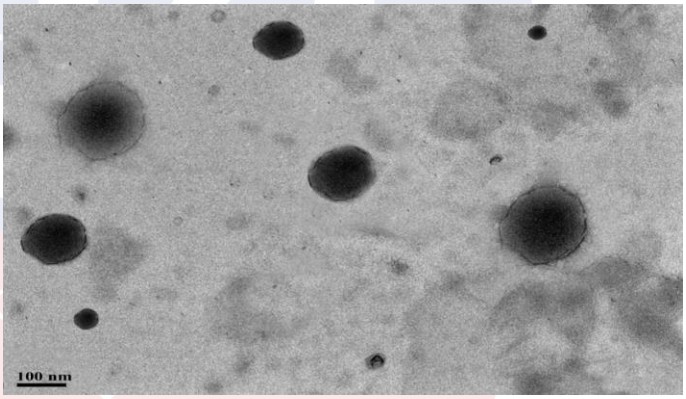


Fig. 3: TEM image of optimized formulation of liposome

Table 4: Results of evaluation of liposomal gel formulations

Code	Drug content (%)		pH	Spreadability (Gm.cm/sec.)	Viscosity (cps)
	Tazarotene	Hydroquinone			
LF1	95.25±2.24	98.25±3.24	7.2±0.024	10.45±0.075	1870±25

LF2	98.15±1.28	99.15±2.28	**7.0±0.035**	**12.32±0.042**	**1895**±33
LF3	97.75±2.12	98.75±2.46	7.1±0.045	11.75±0.049	1875±21

*Average of 03 readings

Drug content from liposomal gel was found to follow the order LF2>LF3> LF1. In drug release study it was observed that the maximum drug release rate was shown in case of LF2 liposomal gel formulation. On the basis of results LF2 was selected as optimized gel formulation based in the drug release and other attributes viz pH,viscosity and Spreadability which was found to be optimum for LF2.

Table 5: *In-vitro* drug release data for Tazarotene Marketed Formulation

Time (min)	Square root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative* % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
5	2.236	0.699	33.56±0.12	1.526	66.44±1.58	1.822
10	3.162	1.000	55.45±0.14	1.744	44.55±1.34	1.649
15	3.873	1.176	68.98±0.25	1.839	31.02±1.08	1.492
20	4.472	1.301	88.98±0.21	1.949	11.02±0.04	1.042
25	5.000	1.398	95.45±0.23	1.980	4.55±0.02	0.658
30	5.477	1.477	98.58±0.21	1.995	1.22±0.02	0.086

*Average of three readings

Table 6: *In-vitro* drug release data of optimized batch LF2 for hydroquinone

Time (min)	Square root of Time (h)1/2	Log Time	Cumulative* % Drug Release	Log Cumulative* % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
5	2.236	0.698	22.25±0.45	1.347	77.75	1.890
10	3.162	1	50.45±0.21	1.702	49.55	1.695
15	3.872	1.176	65.54±0.14	1.816	34.46	1.537
20	4.472	1.301	80.12±0.15	1.903	19.88	1.298
25	5	1.397	90.45±0.21	1.956	9.55	0.980
30	5.477	1.477	98.89±0.56	1.995	1.11	0.045

*Average of 03 readings

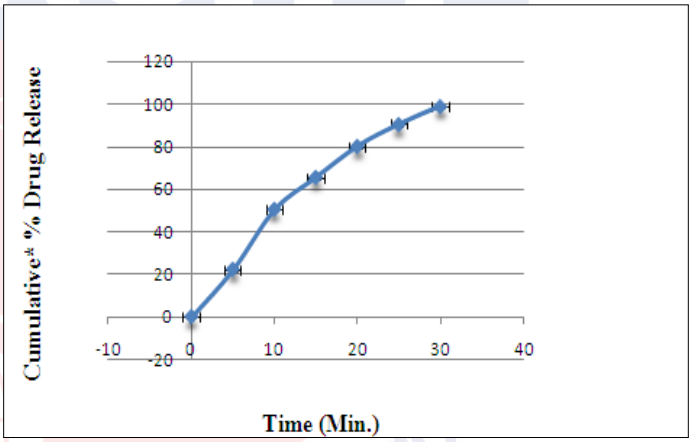


Fig. 4: *In-vitro* drug release data of optimized batch LF2 for hydroquinone

Table 7: *In-vitro* drug release data optimized batch LF2 for tazarotene

Time (h)	Square root of	Log Ti	Cumulative* % Drug	Log Cumulative	Cumulative %	Log Cumulative

	Time(h)$^{1/2}$	me	Release	% Drug Release	Drug Remaining	% Drug Remaining
1	1.000	0.000	23.450± 0.15	1.370	76.550	1.884
2	1.414	0.301	35.450± 0.14	1.550	64.550	1.810
3	1.732	0.477	50.450± 0.25	1.703	49.550	1.695
4	2.000	0.602	63.450± 0.14	1.802	36.550	1.563
6	2.449	0.778	75.450± 0.15	1.878	24.550	1.390
8	2.828	0.903	84.560± 0.36	1.927	15.440	1.189
12	3.464	1.079	98.120± 0.45	1.992	1.880	0.274

*Average of 03 readings

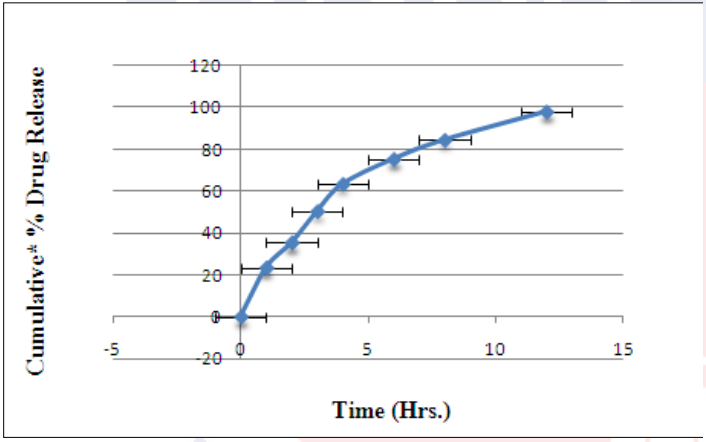


Fig. 5: *In-vitro* drug release data optimized batch LF2 for tazarotene

Table 8: Effect of storage temperature on the vesicle size of drug (Tazarotene) loaded liposomal gel LF2

Time (Days)	Average Vesicle Size (nm)	
	4.0 ± 1°C	28 ± 1ºC
0	168.3	168.2
7	168.5	175.8
14	168.2	179.4
21	168.2	183.7
28	168.5	187.2

*Average of 03 readings

Table 9: Effect of storage temperature on the % drug content of Tazarotene loaded liposomal gel LF2

Time (Days)	Drug Content (%)	
	4.0 ±1°C	28 ± 1ºC
0	99.181 ± 0.020	98.182 ± 0.020
7	98.864± 0.124	98.102 ± 0.123
14	98.426± 0.256	98.012 ± 0.458
21	98.114 ± 0.421	97.014 ± 0.754
28	98.954 ± 0.411	96.425 ± 0.412

*Average of 03 readings

Table 10: Effect of storage temperature on the % drug content of Hydroquinone loaded liposomal gelLF2

Time (Days)	Drug Content (%)	
	4.0 ±1°C	25 ± 1ºC
0	99.211± 0.010	98.125± 0.010
7	98.621± 0.0112	98.101± 0.112
14	98.321± 0.143	98.011± 0.323
21	98.012± 0.322	98.012± 0.643
28	98.824± 0.312	97.324± 0.334

*Average of 03 readings

Stability of optimized (1% w/W LF2) liposomal gel containing drugs was carried out for 4 weeks at 4.0 ± 1°C and 25 ± 1ºC (room temperature). Responses obtained for different parameters for liposomal gel during stability period. Liposomes were found to be reasonably stable in terms of aggregation, fusion and/or vesicle disruption tendencies, over the studied storage period. From results it can be concluded that at room temperature and freeze temperature there was slight decrease in % entrapment efficiency which is insignificant and also an increase in vesicle size was observed

for the optimized batch at 25 ± 1ºC.Thus is due to the fact that a higher temperature the vesicle tent to collide with each other due to increase in the mean kinetic energy and as result they fuse with each other resulting in the increase in the size. During collision, fusion and reformation of the vesicles there is loss of slight amount of the drug because of the bilayer disruption and hence very less or insignificant loss of drug occurs. Result suggests that keeping the liposomal product in refrigeration conditions minimizes stability problems of liposomal gel and hence the best suited temperature for liposomal gel is 4.0 ± 1°C.

Antibacterial activity of liposomal gel

Bacterial cultures: For the studies of antibacterial effect of antibacterial gel formulation, MCC bacterial strains procured from Microbial Culture collection (MTCC), National Centre for Cell Science, Pune, Maharashtra, India. The lyophilized cultures of bacterial strain upon culturing in nutrient broth for 24 hours at 37°±0.5 ° C in an incubator resulted into turbid suspension of activated live bacterial cell ready to be used for antibacterial study. Stored in refrigerated conditions at 4°C as stock culture to be used for further experimentation.

Antibacterial studies: The lawn cultures were prepared with the pathogenic bacterium used under present study by plating the culture onto the solidified agar plates under aseptic hood and potential of the anti bacterial liposomal gel formulation against the bacterium was studied at the concentration of 25, 50 and 100 µg/ml using disc diffusion method.

Antibacterial activity: Anti bacterial activity of the developed formulation was studied evaluated through zone of inhibition study by applying the optimized Liposomal gelat three different concentration (25, 50 and 100 µg/ml) and these were compared with marketed Tazarotene gel at same concentration. The results of anti-bacterial activity are shown in Table **(Yang *et al.*, 2009)**

Table 11: Antibacterial activity of Marketed and optimized gel formulations against *Propionibacterium acnes*

Sample	Zone of Inhibition (mm)		
	25µg/ml	50 µg/ml	100µg/ml
Marketed Gel	15±0.22	20±0.12	22±0.11
Liposomal Gel (LF2)	18±0.12	24±0.10	26±0.20

In present work, liposomal, and marketed gels showed antibacterial activity against *Propionibacterium acnes* with maximum zone of inhibition lying after 24 hours in the range of 18 to 26 mm (Figure).

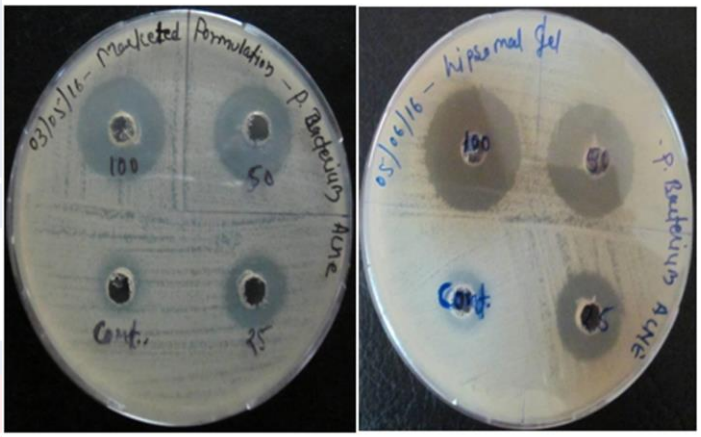


Figure 6: Photograph showing antibacterial activity

Liposomalgel showed greater percentage of inhibition of microbial infection against *Propionibacterium acnes*- On comparison of formulated gels with marketed gel of Tazarotene, Liposomal gel showed greater percentage of inhibition of bacterial infection against *Propionibacterium acnes.* This may be due to the fact that the liposomal gel released the drug in more efficient manner.

Results of *In - Vivo* anti acne activity

Table 12: Effect of Clindamycin (standard), formulation I & II on acne

Sr. No	Group	Mean Thickness ± SEM(excised skin)

1	Normal	1.18 ± 0.09
2	Clindamycin	0.30 ± 0.09***
3	Formulation I (Marketed formulation)	0.65 ± 0.06 **
4	Formulation II (Liposomal gel)	0.45 ± 0.06***

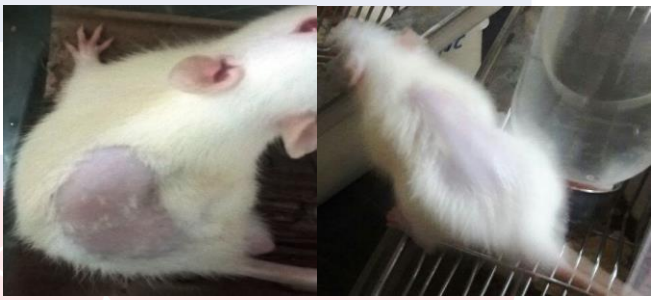
Values expressed as mean ± SEM P<0.05, ** P<0.01, *** P<0.001 as compared to normal*

Acne Induction **Standard Drug**

Formulation I **Formulation II**

Figure 7: Photo Plates of *In - Vivo* anti acne activity

Acne Induction **Standard Drug**



Formulation I **Formulation II**

Figure 8: Photo Plates of *In - Vivo* anti acne activity (after treatment)

Conclusions

Overall results obtained during this work have shown that liposomes could prove interesting carrier for tazarotene in combination with hydroquinone for topical delivery, when appropriate formulations are used. Effectiveness of drug would be increased with liposomal system. Tazarotene in combination with hydroquinone molecules could be successfully entrapped in liposomes with reasonable drug loading. These findings have been seen to support the improved and localized drug action in the skin, thus providing

a better option to deal with skin-cited problems. Hence from results obtained it can be concluded that liposomal gel containing tazarotene in combination with hydroquinone has potential application in topical delivery.

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