Development of Liposomal Gel for Acne Treatment

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Abstract

The aim of the present study was to formulate and evaluate the liposomal gel containing tazarotene and hydroquinone in the treatment of acne. Tazarotene combined with gel hydroquinone to maximize the effect of ophthalmic preparation. The optimization batches 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 formulations (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, 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 theexperimental 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

1. INTRODUCTION

Most favourable therapeutic outcomes necessitate appropriate drug selection. In human body, the skin is a best available space for drug delivery.[1,2] In human body skin covers an area of about 2m and this multi-layered.

In total skin surface consist of 1/1000 of hair follicles in every tetragon centimetre of the skin area. The most gladly reachable. [3,4] The aera of skin where the drug is introduced. Skin are most important barrier for the access of any materials. Transdermal drug delivery crosses the drug at a control systemic circulation. It has intention narrow aera.[5,6]

There are many advantages of transdermal route of conventional routes. It avoid the first pas metabolism effects, activity of extended period and predictable, side effects can be minimizing, the drug utilise goes shorter half-life, improve in physiological and pharmacological. This helps in avoiding the drug level fluctuation also reduce the variabilities. It helps to improve the patient compliances. [7,8]

For the transfer of drugs dermally and transdermal a vesicular system of drug delivery is introduced. For overcome this problem liposomes are use. The bilayer lipid vesicles, phospholipids and cholesterols.[9,10] Bangham and colleagues discover the liposomes by drug delivery system. The solvents are separated from each other. These solvents are closed, spherical. The exterior envelops of a liposome are allowed to passes the drug by lipophilic skin. This treatment is use for the both local and internal skin disorders. Cosmetic formulation has shown the systemic effects.[11,12]

2.MATERIAL AND METHODS

2.1 Pre-formulation Studies

2.1.1 Physical appearance:

Organoleptic properties are used for the examination of drugs (Tazarotene and Hydroquinone).

2.1.2 Determination of wavelength maxima (λmax):

Around 10mg of each drug are weigh and dissolved into 100ml of PBS (pH 7.4) in a 100ml of volumetric flask. 1ml of solution are pipette out and transfer 10ml of flask and volume was make up with PBS (pH 7.4). UV/Vis double beam spectrophotometer is use for the scanning the solution (200-400nm).

2.1.3 Preparation of Standard Stock Solution:

10mg of drugs are weigh and dissolved into 10ml of PBS (pH 7.4) and makeup the volume and formed a stock solution of 1000 ppm or µg/ml.

2.1.4 Calibration curve of Tazarotene

Form the stock solution(1mg) is taken out and dissolved into 10ml of buffer solution. 0.1, 0.2, 0.3, 0.4- and 0.5-ml solutions are made and transfer into the flask. At 351nm the absorbance of tazarotene is recorded. Using linear regression concentration straight is best obtained.

2.1.5 Calibration curve of Hydroquinone

Stock solution(1mg) is taken out and dissolved into 10ml of buffer solution. 1.0, 2.0, 3.0, 4.0- and 5.0-ml solutions are made and transfer into the flask. At 288nm the absorbance of hydroquinone is recorded. Using linear regression concentration straight is best obtained.

2.1.6 FTIR spectroscopy

By using KBr method, physical mixture of drug and pure drug is determined. The base line is made by the potassium bromide pellet dried. The IR compartment is mounted and scanned at 4000 cm^{-1} to 400 cm^{-1}.

2.1.7 Melting point

For the determination of purity of drug this parameter is used. Open capillary method is for the determination of melting point. At 5ºC the sample amount (2-5mg) was put into one side fused capillary tube. The melting of drug is calculated.

2.1.8 pH measurement

Digital pH meter is use for the determination of pH. 1gm of sample were weigh and dissolved into 5ml of ethanol by using sonicator. After that the sample was filter and the pH was measured by pH meter.

2.1.9 Flow properties

The angle of repose, Carr's index and haussner ratio is use for the characterization of flow property of powder. This funnel was fixed in such a way that the lower tip was at a height of 2cm from the hard surface. In cylindrical glass funnel the drug powder was poured and mark up to 10ml. The excessive blend is remove by spatula and the pellet is put into cylinder and the volume was calculated.

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

Table 1. flow property

2.1.10 Solubility study

This study was performed in distilled water, 0.1N HCl, alcohol, methanol, chloroform, acetone, dimethyl sulphoxide (DMSO), Phosphate buffer saline pH 7.4 at room temperature (25± 20 C).

Descriptive	Parts of solvent required for Parts of soluble
Very soluble	Less than 1
Freely soluble	From 1to 10
Soluble	From 10 to 30
slightly soluble	From 100 to1000
Very slightly soluble	From 1000 to 10000
Practically	Insoluble10000 or more

Table 2. Ranges for solubility

2.1.11 Partition coefficient

The un-ionized solute was dissolved into solvents for the ratio of concentrations (log *P*). this is also known as lipophilicity. It is described in partition coefficients.

For examination of Partition coefficient of ketoconazole n-Octanol: water system. In a separating funnel 5mg of drug and 10ml of octanol with water is added. Shaked the apparatus for 2-3 hours for equilibration on rotatory shaker. The drug concentration in octanol was estimated by spectrophotometrically and prepared the calibration curve. The drug partition coefficient was calculated by: -

Partition coefficient K $= \dfrac{\text{Amount of drug in organic layer}}{\text{Amount of drug in aqueous layer}}$

2.1.12 Loss on Drying

IR moisture is use for the determination of loss of drying. 5gm of drug sample was measured and weigh and the temperature are set for 5 minutes. Calculate the moisture of percentage.

2.1.13 Drug –Excipients compatibility study

differential scanning calorimeter is use for the determination of thermograms. 5-10mg of drug is weigh and put it in hermetically sealed bottomed aluminium pans. Over 50 to 400^0C temperature the sample were heated in presence of nitrogen for 10min.

2.2 Preparation, Optimization and Characterization of liposomal

2.2.1 Liposome Preparation

Drug: SPC:CHOL ratio was determined with vesicular size and drug entrapment efficiency. At $40^0 \pm 0.5^0$C a mixture of chloroform: methanol (2:1) were

For the screening of formulation and process variables a regular 2^3 factor design was use for the development of liposome.

evaporated and the lipid film from the bottom of the flask were collected. After that the lipid film at a temperature of $37^0 \pm 0.5^0$C were hydrated. Sonication was done at 4^0C for 30sec in 3 cycle.

2.2.2 Influential variables screening

Factor	level	
	Low (-1)	**High (+1)**
Amount of Lecithin (mg)	100	200
Amount of Cholesterol (mg)	20	50
Rotation Speed (RPM)	100	200

Table 3. list of variables

Run	Batch No.	Lecithin (mg)	Cholesterol (mg)	Rotation Speed (rpm)
1	TL1	100	30	200
2	TL2	100	40	200
3	TL3	200	30	100
4	TL4	200	30	200
5	TL5	200	40	200
6	TL6	100	30	100
7	TL7	100	40	100
8	TL8	200	40	100

Table 4. Composition of liposome

2.2.3 EVALUATION OF LIPOSOME

2.2.3.1 Vesicle size:

Particle size analyser is use for the determination of vesicular size.

2.2.3.2 Entrapment efficiency

By using ultra centrifuge method, drug (tazarotene and hydroquinone) was estimate in liposome. A 10ml centrifuge tube was use for the transfer of liposomal suspensions. 5ml of water is use for the dilution of this suspension and rotate at 2000RPM for 10mintues. By using this method separation of undissolved drug is done. Aggregation of liposome in presence of protamine and after separation was done at 15,000RPM about 15-20minutes. Supernatant and sediment are separated out. After that in 5ml water sediment is dissolved. The drug entrapment and unentraped are analysed by using supernatant and liposomes by calibration curve method using U.V. Vis. Spectroscopy.

2.2.3.3 TEM analysis

TEM is use for the determination of surface morphology. A sample drop was placed on carbon coated copper grid for 15 min.

2.3 Preparation of gels

In 100ml of water, 0.5g of Carbopol was weigh and dissolved and mild stirring was done. Put it 24hours to obtained 0.5% of gel. After that 2ml of glycerine is added to the gel. methyl Paraben and Propyl Paraben are added as a preservative.

Formulation	Carbopol (%)
LF1	0.5
LF2	1.0
LF3	2.0

Table 5. different gel composition

2.4 LIPOSOMAL GEL PREPARATION

10mg of liposomal formulation was weigh and dissolved into 10ml of ethanol and at 6000RPM it was centrifuged for 20minutes.

At 25RPM this incorporated 0.1% tazarotene and 4% hydroquinone were slowly mechanical achieved.

2.4.1 Evaluation of Gel

2.4.1.1 Determination of pH

In 10ml of beaker 50gm of gel formulation was transferred and the pH was determined. pH ranges from 3-9 is use for the treatment of skin infection.

2.4.1.2 Spreadability

On the bases on slip and drag characteristics the liposomal gel spreadability was determined. Two glass slide was taken. Formula S=ml/t is use for the determination of Spreadability.

Where S= Spreadability

M= upper slide pan weight

T= time take

L= distance travel

2.4.1.3 Measurement of viscosity

Brookfield viscometer is use for the determination of viscosity of gels.

2.4.1.4 Drug content

4mg of drug is weighed and transfer to 10ml volumetric flask. Volume was made up for obtaining the concentration of 400µg/ml. After that filtration was done by Whatmann filter paper. At 315nm and 288nm the absorbance of sample was done.

2.4.1.5 Drug Release Kinetics

Dissolution data were calculated by different type of models such as zero-order, first order, Higuchi equations and Peppas equations. The bilayer system was shown by zero-order or may be first order kinetics. Higuchi and peppas equation are used for the drug release mechanism.

1. Zero order release kinetics:

It may be defined as a relationship between the drug release and time.

$Q_t = Q_o + k_o t$

Where Q_t = at time t the fraction of drug release

Q_o= initial amount of drug in the solution

k_o= rate constant

2. First order release kinetics:

In dissolution process the surface aera may be decreased with time. By this the drug may release very slowly. equation which is use for this is:

$\text{Log } Q_t = \text{ log } Q_o + \frac{k_1 t}{2.303}$

Where Q = at time t the fraction of drug release

K_1= rate constant

3. Higuchi equation:

$Q = K_H t^{1/2}$

Where, K_H= rate constant

Q = at time t the fraction of drug release

This equation is based on Fick's law.

4. Korsmeyer and Peppas equation:

$$F = (M_t/M) = K_m t^n$$

M_t/M = drug released

T= release time

N= diffusional exponent for drug release

3. RESULT AND DISCUSSION

3.1 Organoleptic study

Parameter	Tazarotene	Hydroquinone
Colour	Light yellow powder	White crystalline powder
Odor	Odorless	Odorless
Taste	Tasteless	Tasteless

Table 1. Organoleptic study

3.2 Determination of wavelength maxima in PBS pH 7.4

For the estimation of drug (Tazarotene and hydroquinone) different types parameters are used.

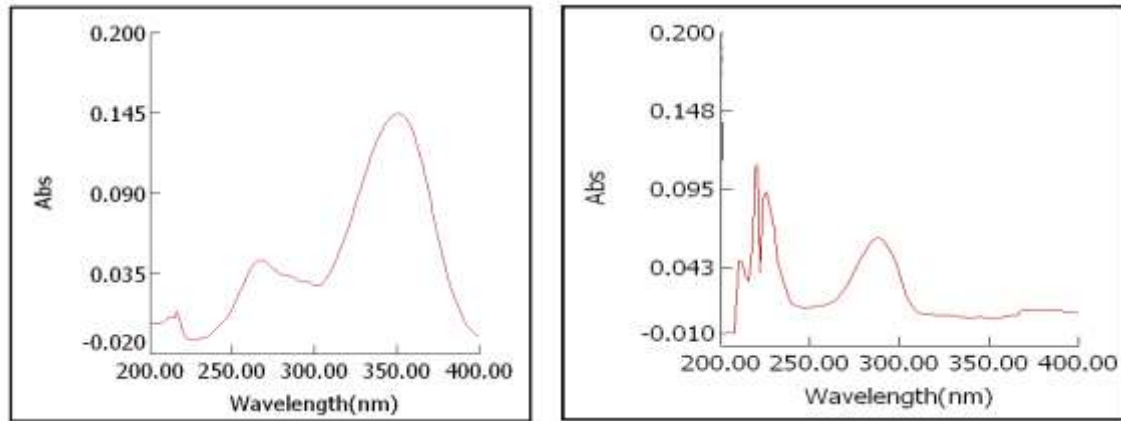### 3.2.1 Determination of λ_{max}

Figure 1. Scanned Wavelength (Tazarotene and Hydroquinone) with PBS pH 7.4

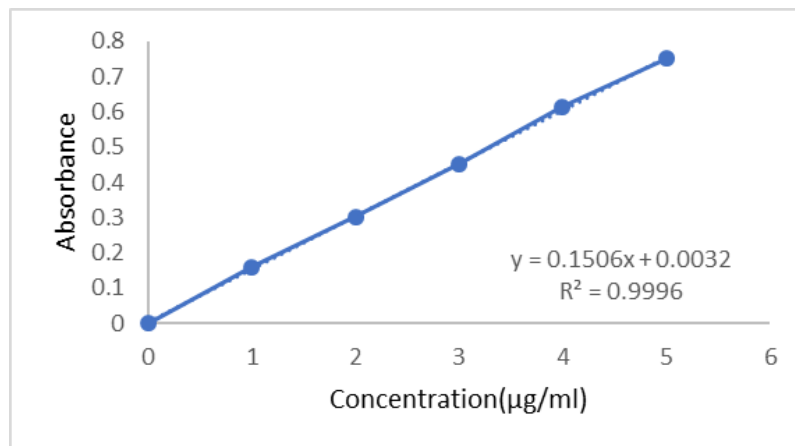### 3.2.2 Calibration curve of drug (Tazarotene and Hydroquinone)
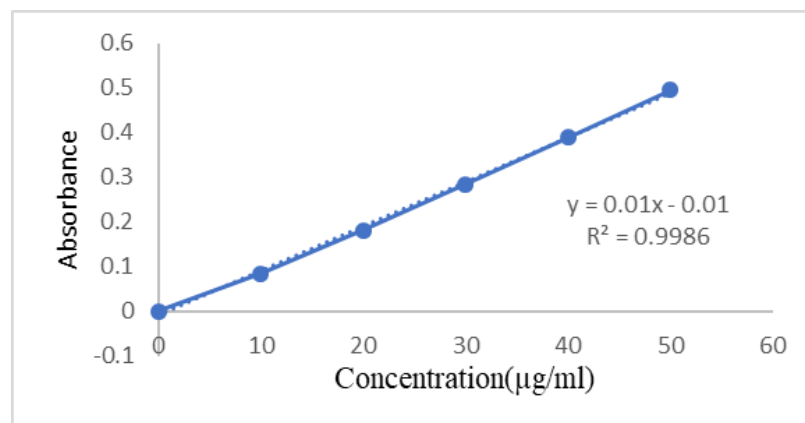

Figure 2. calibration curve of tazarotene

Figure 3. calibration curve of tazarotene
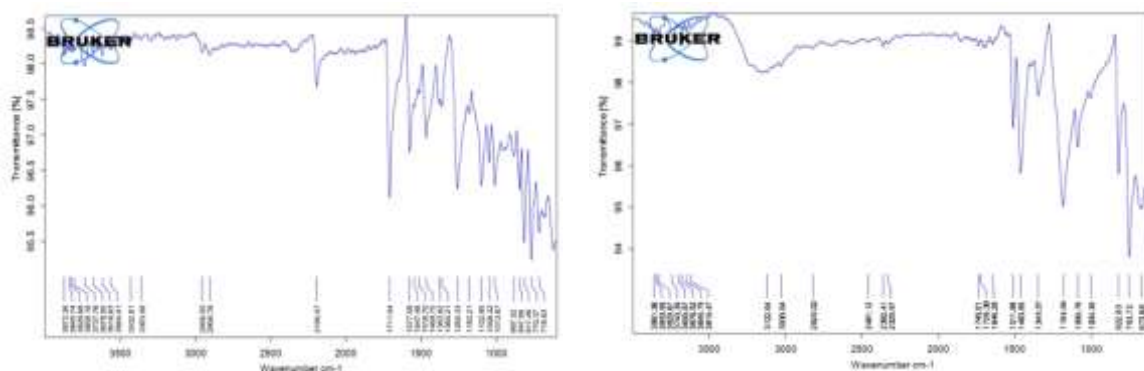
3.2.3 Fourier-Transform Infra-Red Spectroscopy (FTIR)

Figure 4. FTIR of hydroquinone and tazarotene

3.2.4 Melting point determination

The drug sample was done by melting point. It proves the percentage purity of the sample. A small amount of impurity may lower the melting point. The melting point of Tazarotene and hydroquinone was found to be 95±1.2°C and 172±2°C.

3.2.5 pH of drug solution

By using digital pH meter, the pH of drug was determined and it was found to be 6.9±0.002 and 7.2±0.003.

3.2.6 Flow properties:

Parameter	Tazarotene	Hydroquinone
Angle of Repose	27.08o	33.15o
Carr' index (%)	0.39	0.66
Haussner's Ratio	1.10	1.15

Table 9. flow property

2.2.7 Solubility studies

At different solvent and at different room temperature solubility study was performed. Drugs (Tazarotene and hydroquinone) are freely soluble in ethanol, methanol, chloroform and slightly soluble in 0.1N HCL, acetone and insoluble in water.

Solvent	Solubility (mg/ml)	
	Tazarotene	Hydroquinone
Distilled Water	0.00075	11.26
0.1 N Hydrochloric acid	16.36	22.56
Ethanol	17.57	25.65
Methanol	26.26	31.25
Chloroform	18.56	5.26
Acetone	15.58	8.56
DMSO	35.15	15.56
Phosphate buffer pH 7.4	8.12	22.15
Phosphate buffer pH 6.8	9.25	25.37

Table 10. Solubility Study

2.2.8 Partition coefficient determination

In this process, transfer of drug in aqueous and non-aqueous layer is find out. This was use for the drug concentration in either layer. The drugs (tazarotene and hydroquinone) are found to be   1.21±0.001 and 0.85±0.002

2.2.9 Loss on drying:

The loss on drying percentage of Tazarotene and hydroquinone was found to be 0.68±0.013% w/w and 0.45±0.013% w/w.

2.2.10 Drug-Excipients compatibility study:

The melting point of drugs is found to 95^{0}C and 172^{0}C. In DSC analysis the drug and cholesterol was found to be 40^0 C/ 75% RH for 30 days.
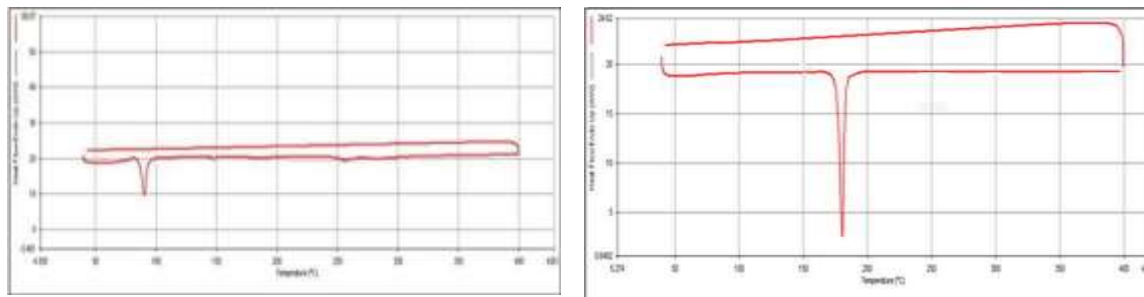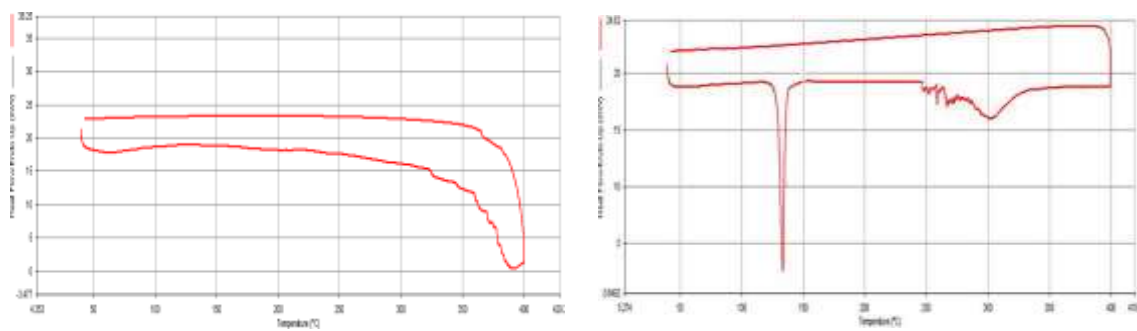

Figure 5. DSC study of Tazarotene and hydroquinone

Figure 6. DSC study of cholesterol   DSC study of Drug+ All

2.3 Evaluation of formulations

Formulation	Vesicle size (nm)	Zeta Potential (mV)	Entrapment efficiency (%)	Polydispersity Index (PDI±SD)
TL1	169.8	-33.6	59.6±0.68	0.411
TL2	278.6	29.3	58.62±1.58	0.229
TL3	450.2	28.3	64.65±0.96	0.321
TL4	410.2	19.6	65.65±3.60	0.232
TL5	559.2	-33.6	65.36±2.97	0.301
TL6	187.6	-39.5	66.65±2.69	0.221
TL7	320.1	30.5	67.89±3.65	0.839
TL8	805.6	32.5	68.36±2.78	0.628

Table 11. Evaluations of Liposomal Formulations
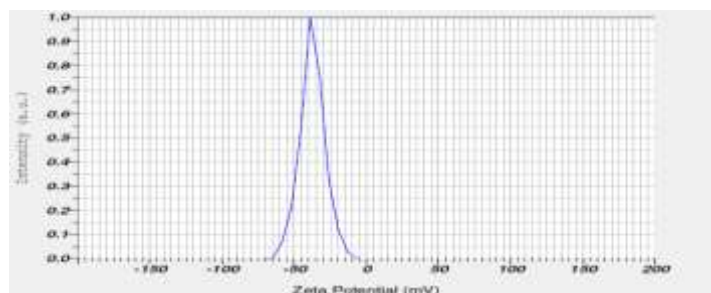
2.3.1 Zeta potential study

Figure 7. Zeta potential
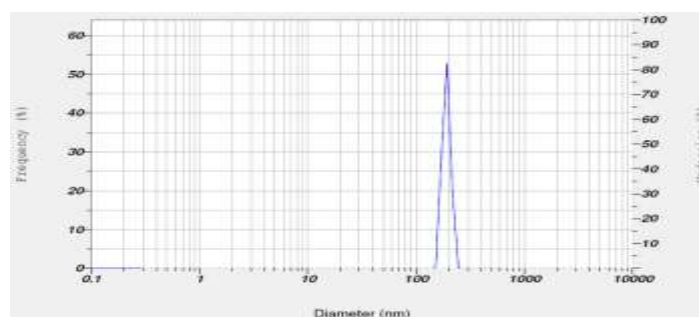
2.3.2 Vesicle size study

Figure 8. Vesicle size

2.4 Evaluation of liposomal gel
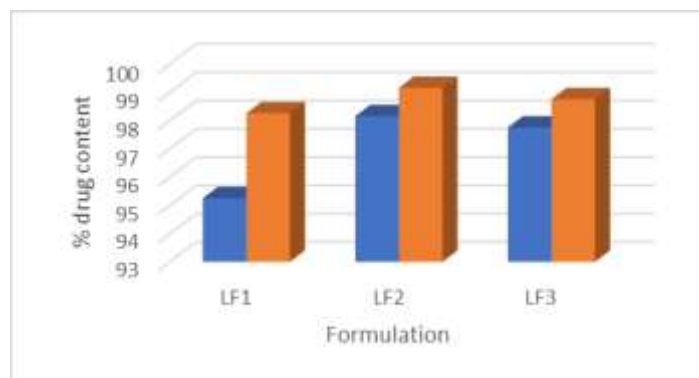
2.4.1 Result of assay of gel formulation

Figure 9. % Drug content of liposomal formulations

2.4.2 pH

pH plays a most important role in transdermal drug delivery system. The prepared liposomal gel is acceptable and within the limit 7.0-7.2.

2.4.3 Spreadability

Speadability was determined by modified apparatus. The speadeability is in the range between 10.45-12.32gms. cm. /sec. gel has optimum Spreadability sue to the high and very low values.

2.4.4 Viscosity measurements

Brookfield viscometer is use for the determination of viscosity. Helipath stand is use for the measurement of viscosity.
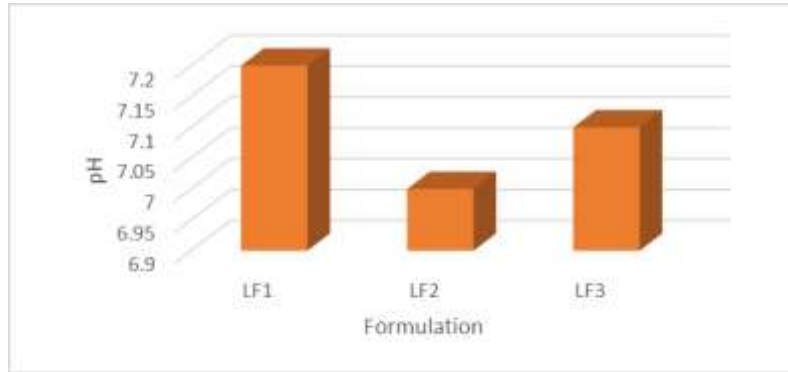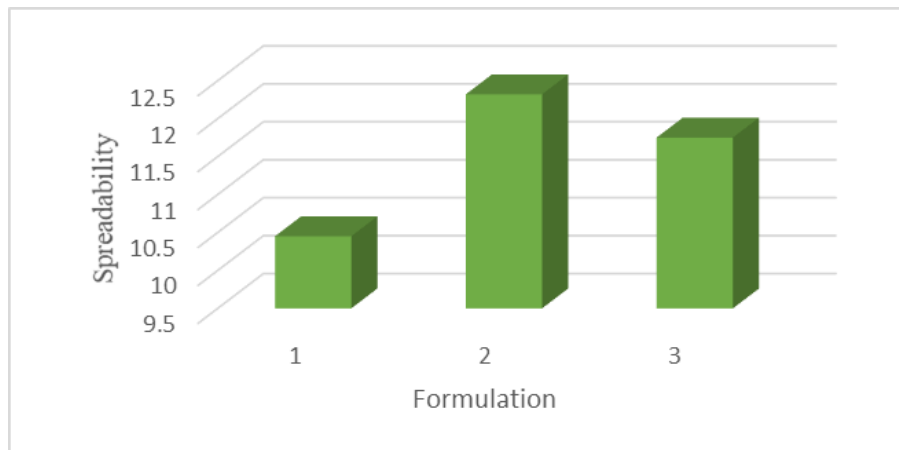
Figure 10. pH of liposomal formulations
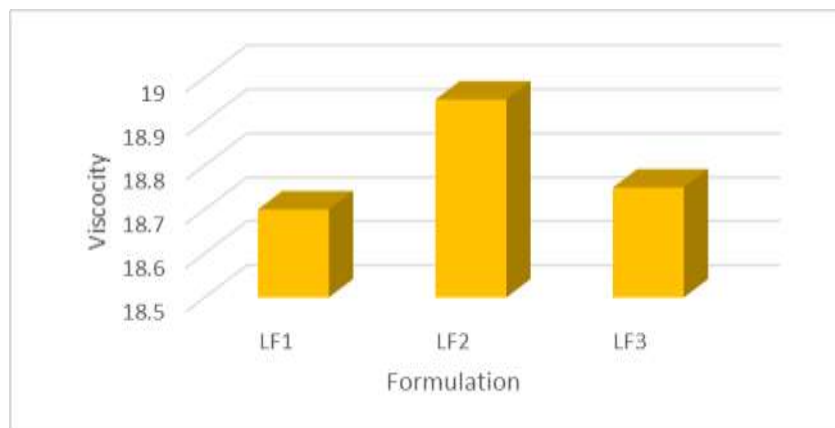

Figure 11. Spreadability of liposomal formulations

Figure 12. Viscosity of liposomal formulations
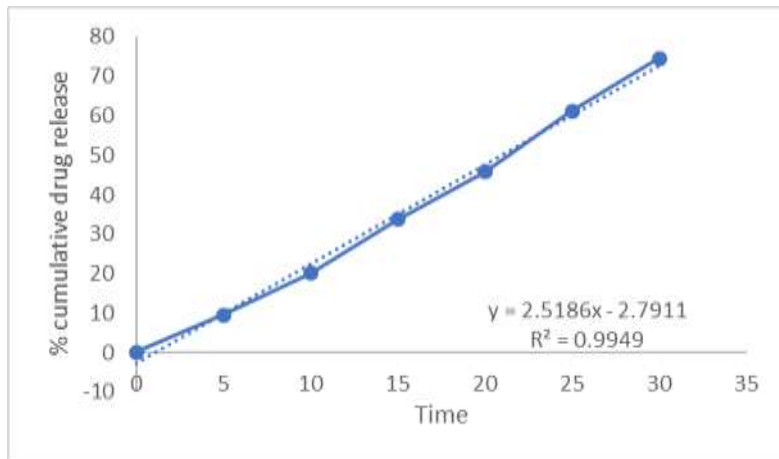
2.4.5 Kinetic Analysis of Release
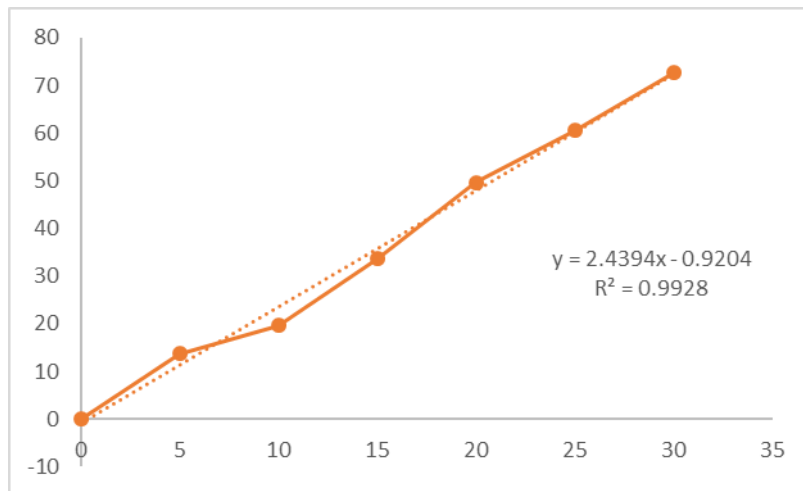

Figure 13. Zero order kinetics

$y = 2.5186x - 2.7911$
$R^2 = 0.9949$

Figure 14. first order kinetics

$y = 2.4394x - 0.9204$
$R^2 = 0.9928$

Figure 15. Higuchi plot

$y = 1.4028x - 1.0246$
$R^2 = 0.9979$

The chart shows a plot with the equation:

$$y = 1.7429x - 1.5311$$
$$R^2 = 0.9962$$
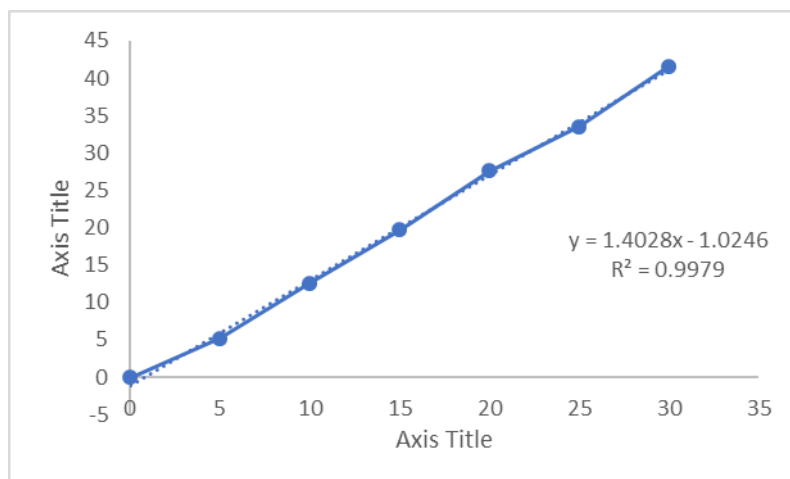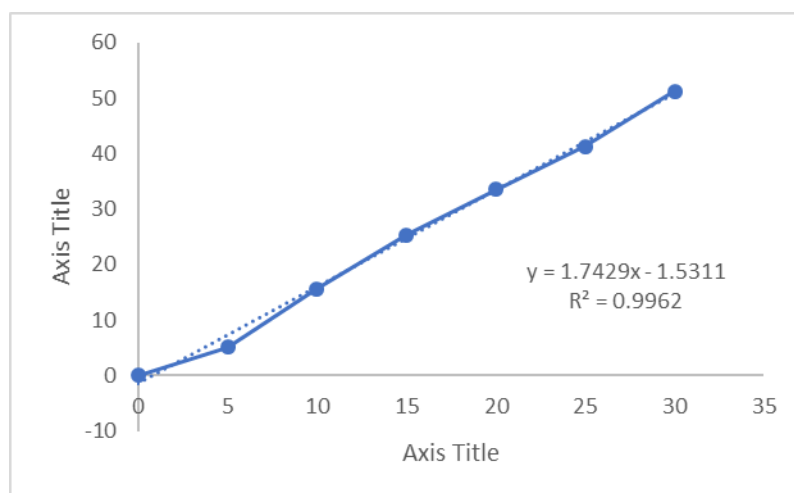
Figure 16. Peppa's plot

4. SUMMARY & CONCLUSION

The present study shows the formulation and evaluation of liposomal gel (hydroquinone & tazarotene) for the treatment of acne.

Two drug is selected for the study (tazarotene and hydroquinone). Tazarotene is vitamin A similar and hydroquinone is chemical compounds which inhibit the conversion of dopa to tyrosinase enzyme. Both the drug is collected from reputed company as a gift sample. Various parameters are used for the determination of effectiveness. The solubility of drug is determined with different types of solvents (HCL, ethanol, methanol, chloroform, acetone, PBS).

The partition coefficient of the drugs are found to be 1.21 ± 0.001 and 0.85 ± 0.002. the pH of the drug is with in the range.

The gel formulated consists of the Tazarotene loaded liposomes which are then included in the gel containing hydroquinone. The gel formulation has a mixer of Tazarotene and hydroquinone. This may go into the deepest layer of the skin. Cholesterol is use for the bilayer of both the drugs. It increases the viscosity of the bilayers.

TEM study is use for the study of morphology. In which the sample was put into carbon coated copper grid and stained with 1% aqueous solution of phosphotungustic acid. TEM revealed that liposomes have mean size of 100-500 nm.

1% & 2% Carbopol gels were prepared. The optimized liposome formulation. The pH is within the limit 7.0 to7.2.

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