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Formulation and Evaluation of Liposomal Gel for the Treatment of Acne

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ABSTRACT

The main aim of the thesis is to formulation and evaluation of liposomal gel for the treatment of acne. Acne is the most uncurable disorder now a day. It was stay up to years. Topical route is more preferable for the treatment of acne. The drug is collected from reputed company as a gift sample. Various parameters are used for the determination of effectiveness. The gel formulated consists of the dapsone loaded liposomes which are then included in the gel. TEM study is use for the study of morphology. Stability study shows that the drug is stable at different temperature. So, dapsone is useful against acne.

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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 area of skin where the drug is introduced. Skin is 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 [13, 14, 15]

Physical appearance: Organoleptic properties are used for the examination of drugs (Dapsone).

Determination of wavelength maxima (λ_{max}): 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).

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.

Calibration curve of dapsone: 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. Standard solution of 1, 2, 3, 4, 5 µg/ml concentration is used. At 292nm the absorbance of Dapsone is recorded. Using linear regression concentration straight is best obtained.

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 potassium bromide-and sample pellet of l mm diameter was prepared by grinding 3-5 mg of physical mixture of drug-excipients with 100-150 mg of potassium bromide in pressure compression machine. The IR compartment is mounted and scanned at 4000 cm-1 to 400 cm^{-1}.

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.

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.

Flow properties: The angle of repose, Carr's index and haussner ratio is use for the characterization of flow property of powder. Angle of repose is determined by pouring a sample through the side of the funnel. 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 removed by spatula and the pellet is put into cylinder and the volume was calculated.

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± 2 °C).

Table No. 1. Ranges for solubility

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

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 dapsone 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 = (Amount of drug in organic layer)/(Amount of drug in aqueous layer)

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 by 100°C to 105°C for 5 minutes. Calculate the moisture of percentage.

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 °C temperature the sample were heated in presence of nitrogen for 10min.

2.2 Preparation, Optimization and Characterization of liposomal [16-19]

Liposome Preparation: By using lipid film hydration method liposome are prepared. Drug: SPC:CHOL ratio was determined with vesicular size and drug entrapment efficiency. At

400±0.50C a mixture of chloroform: methanol (2:1) were evaporated and the lipid film from the bottom of the flask were collected. After that the lipid film were hydrated with PBS (pH 7.4) at a temperature of 37±0.5 °C. Sonication was done at 40C for 30sec in 3 cycle.

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

Table No. 2. List of variables

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

Table No. 3. Composition of liposome

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

2.3 Evaluation of Liposome: [20-22]

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

Entrapment efficiency: By using ultra centrifuge method, drug (dapsone) 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 10 minutes. By

using this method separation of undissolved drug is done. Aggregation of liposome in presence of protamine and after separation was done at 15,000 RPM about 15-20 minutes. Supernatant and sediment are separated out. After that in 5ml water sediment is dissolved. The drug entrapment and unentrapped are analysed by using supernatant and liposomes by calibration curve method using U.V. Vis. Spectroscopy.

TEM analysis: TEM is use for the determination of surface morphology. A sample drop was placed on carbon coated copper grid for 15 min. this was negatively stained with 1% solution of phosphotungustic acid.

2.4 PREPARATION OF GELS:[23, 24]

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.

Table No. 4. Different gel composition

Formulation	Carbopol (%)
LF1	0.5
LF2	1.0
LF3	2.0

2.5 Liposomal Gel Preparation:[25, 26]

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

At 25RPM this incorporated 4% of drug were slowly mechanical achieved.

2.6 EVALUATION OF GEL [27]

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

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.

$$\text{Spreadability: } S = M \times L\ T$$

Where S= Spreadability

M= upper slide pan weight

T= time take

L= distance travel

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

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 292nm the absorbance of sample was done.

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 Qt = at time t the fraction of drug release

Qo= initial amount of drug in the solution

ko= 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:

$$\text{Log } Q_t = \log Q_o + \frac{k1t}{2.303}$$

Where Q = at time t the fraction of drug release

K1= rate constant

3. Higuchi equation:

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

Where, KH= rate constant

Q = at time t the fraction of drug release

This equation is based on Fick's law.

4. Korsmeyer and Peppas equation:

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

Mt/M = drug released

T= release time

N= diffusional exponent for drug release

2.7 Stability studies: Dapsone loaded liposomes are accelerated to stability studies with a storage condition of 4 ± 1 °C at a temperature of 25 °C with 60±5%RH humidity. After a period of time 28days the sample is analysis. [28]

3. RESULTS AND OBSERVATION

3.1 Pre-formulation Study

Table No. 5: Organoleptic study

Parameter	**Dapsone**
Colour	White crystalline powder
Odour	Odourless
Taste	Tasteless

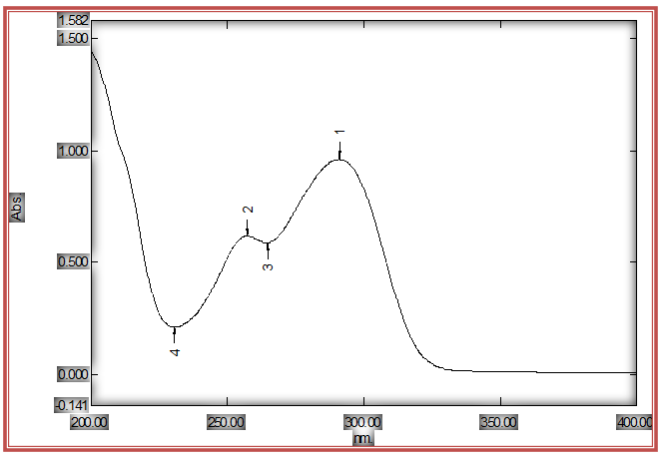

Figure No. 1. Scanned Wavelength (dapsone) with PBS pH 7.4

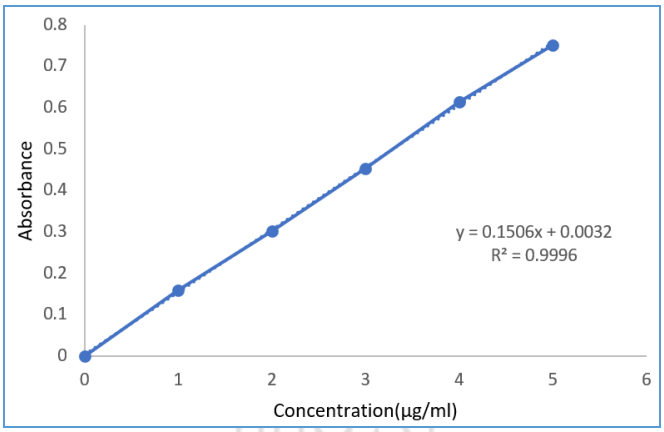

Figure No. 2. Calibration curve of Dapsone

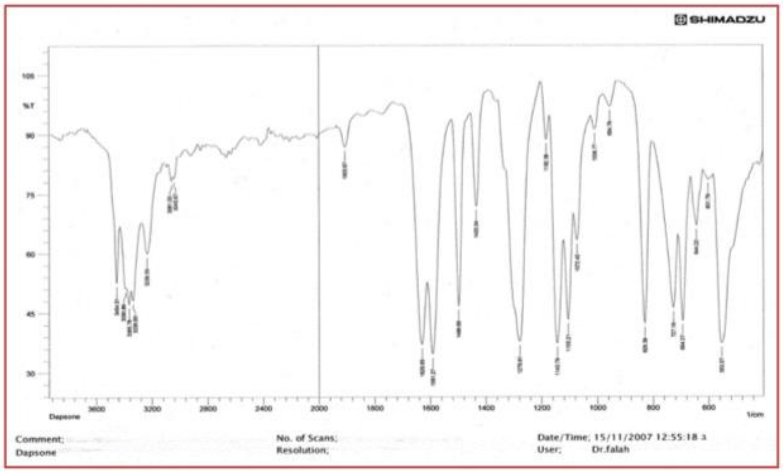

Figure No. 3. FTIR of Dapsone

Melting point determination: It proves the percentage purity of the sample. A small amount of impurity may lower the melting point. The melting point of dapsone was found to be 179°C.

pH of drug solution: By using digital pH meter, the pH of drug was determined and it was found to be 6.8.

Table No. 6: Flow properties

Parameter	**Dapsone**
Angle of Repose	27.08
Carr' index (%)	0.39
Haussner's Ratio	1.10

Table No. 7. Solubility Study

Solvent	**Solubility (mg/ml) dapsone**
Distilled Water	0.00075
0.1 N Hydrochloric acid	16.36
Ethanol	17.57
Methanol	26.26
Chloroform	18.56
Acetone	15.58
DMSO	35.15
Phosphate buffer pH 7.4	8.12
Phosphate buffer pH 6.8	9.25

Partition coefficient determination: This was use for the drug concentration in either layer. The drugs (dapsone) are found to be 1.21±0.001.

Loss on drying: The loss on drying percentage of dapsone was found to be 0.5±0.013% w/w.

Drug-Excipients compatibility study: The melting point of drugs is found to 179 °C. In DSC analysis the drug and cholesterol were found to be 40 °C/ 75% RH for 30 days.

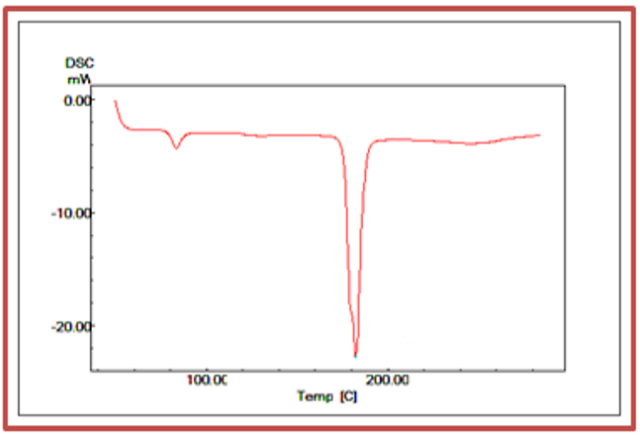

Figure No. 4. DSC study of Dapsone

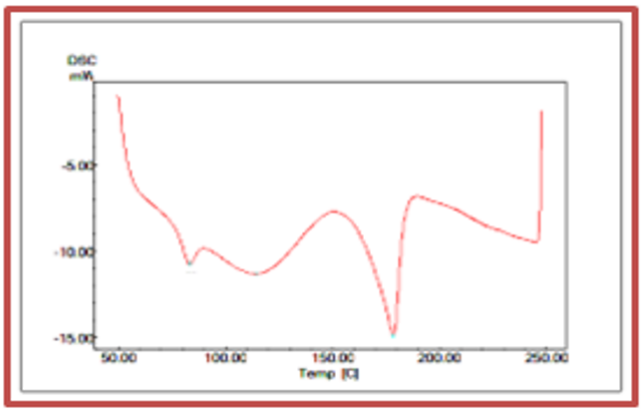

Figure No. 5. DSC study of cholesterol

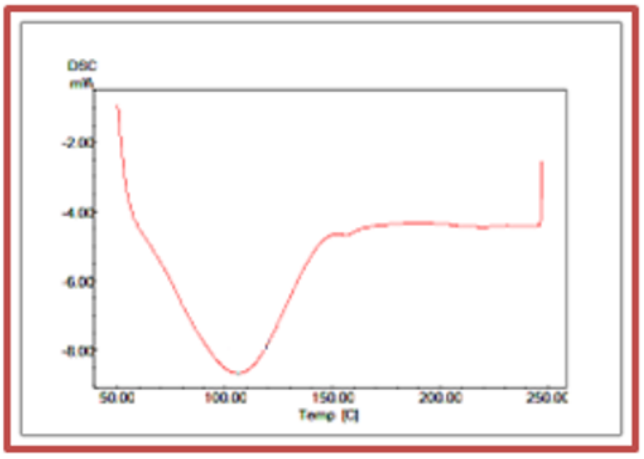

Figure No. 6. DSC study of Drug+ All

Table No. 8. Evaluations of Liposomal Formulations

Formulation	Vesicle size (nm)	Zeta Potential (mV)	Entrapment efficiency(%)	Polydispersity Index (PDI±SD)
TL1	170.8	-33.6	59.6±0.68	0.411
TL2	258.6	29.3	58.62±1.58	0.229
TL3	449.2	28.3	64.65±0.96	0.321
TL4	425.2	19.6	65.65±3.60	0.232
TL5	575.2	-33.6	65.36±2.97	0.301
TL6	195.6	-39.5	66.65±2.69	0.221
TL7	378.1	30.5	67.89±3.65	0.839
TL8	879.6	32.5	68.36±2.78	0.628

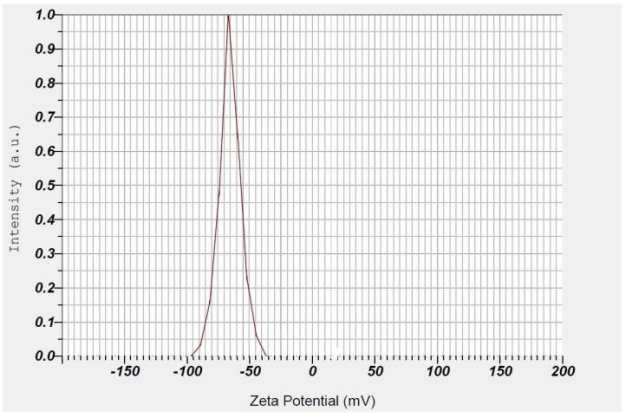

Figure No. 7. Zeta potential

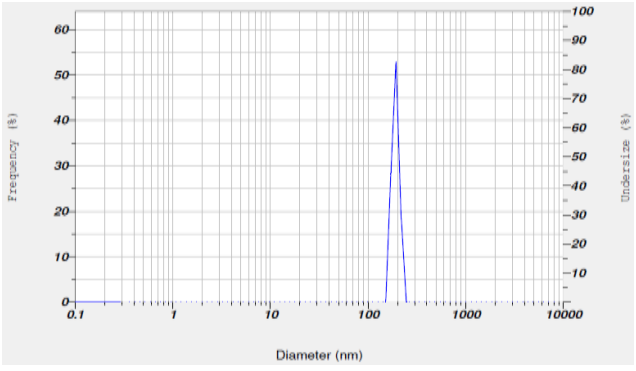

Figure No. 8. Vesicle size

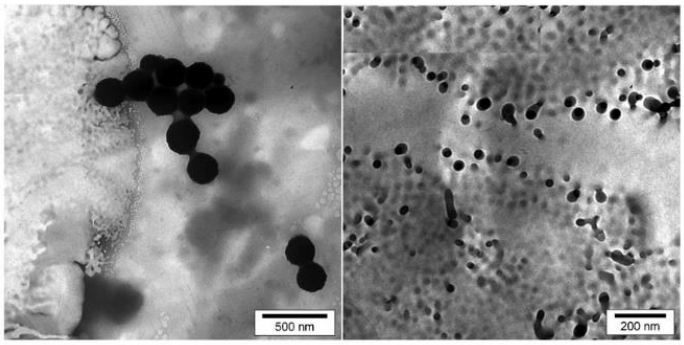

Figure No. 9. TEM analysis

EVALUATION OF GEL:

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.

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

Viscosity measurements: Brookfield viscometer is use for the determination of viscosity. Helipath stand is use for the measurement of viscosity.

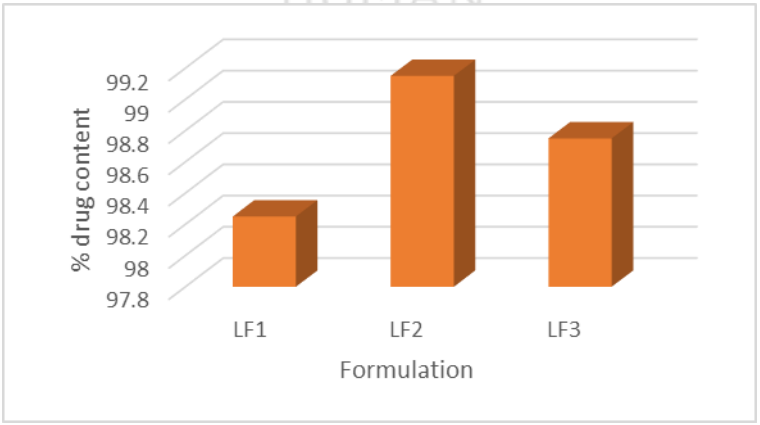

Figure No. 10. % Drug content of liposomal formulations

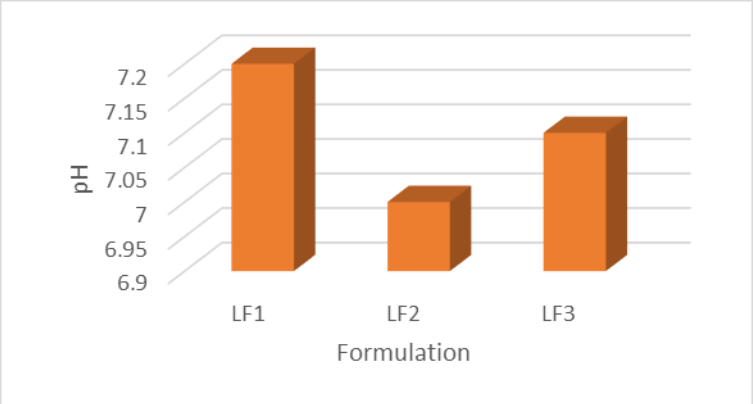

Figure No. 11. pH of liposomal formulations

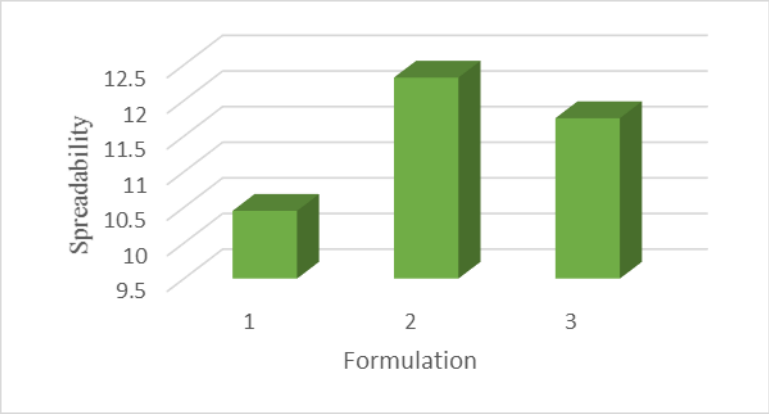

Figure No. 12. Spreadability of liposomal formulations

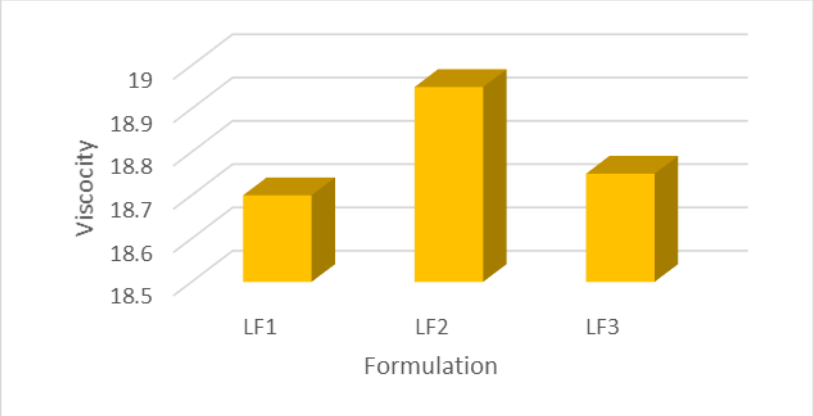

Figure No. 13. Viscosity of liposomal formulations

Kinetic Analysis of Release: Zero order, first order, Higuchi and Peppas model is use for the calculation of release data. These data are interpreted due to the changes in values of regressions coefficients. in-vitro release follows first order kinetics followed by Peppas model. It hep to release mechanism for explanation. Calculated the value of slope and the

result was found to be <0.89. this was suggested that the anomalous diffusion was coupling the erosion mechanism.

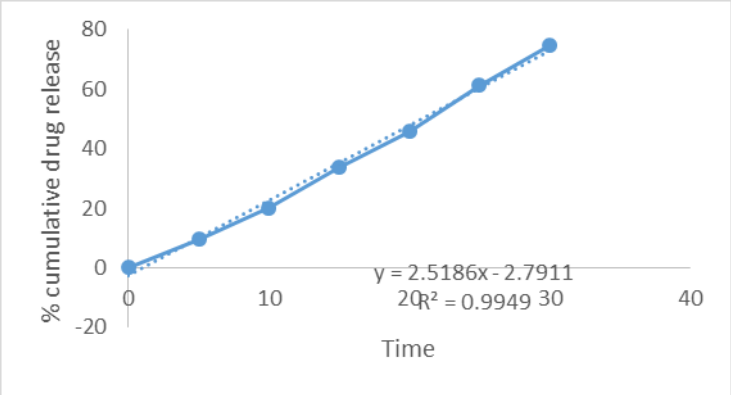

Figure No. 14. Zero order kinetics

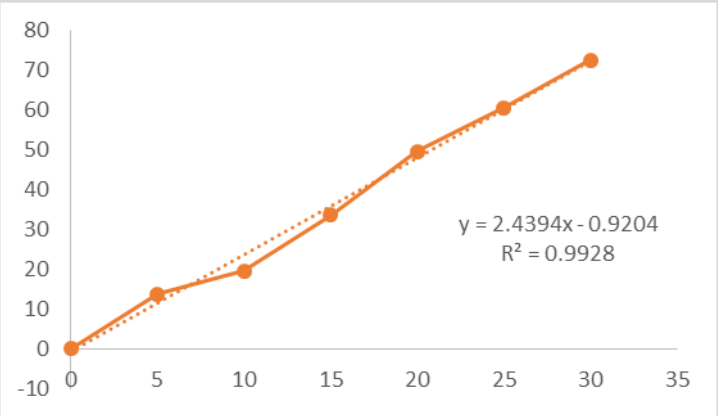

Figure No. 15. first order kinetics

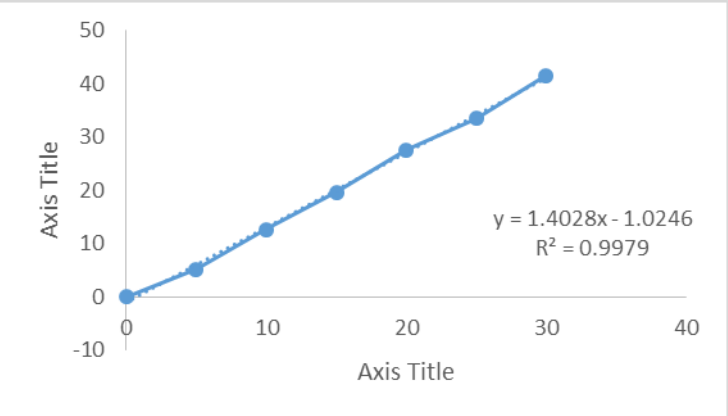

Figure No. 16. Higuchi plot

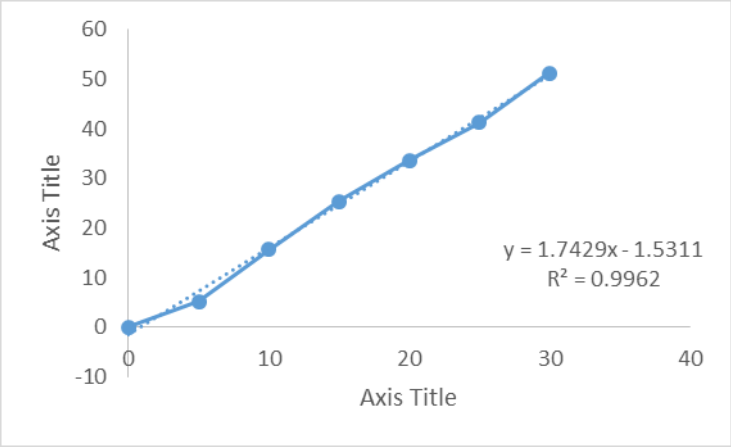

Figure No. 17. Peppa's plot

Table No. 9: Stability Studies

Time(days)	**Average size (nm)**	
	4.0 ±1°C	25 ± 1ºC
0	168.3	168.2
7	168.5	175.8
14	159.2	179.4
21	156.2	183.7
28	153.5	187.2

4. DISCUSSION AND CONCLUSION

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

Drug is selected for the study (Dapsone). 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 was found to be 1.21±0.001. the pH of the drug is within the range.

The gel formulated consists of the dapsone loaded liposomes which are then included in the gel. 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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