Acne has been often been nightmare to the majority of the population especially teen aged girls. The same has also impacts on the social life of a person once he/she is affected by Acne. There are varieties of the dosage forms that have been or are being utilized to cure the same. However still drug delivery aspects are being deployed to make the therapy more efficient. In the clinical management of acne, topical formulations are the preferred because of the ease associated with their application. In addition, combination therapy often proves more efficacious and better tolerated than mono therapy with a single drug

Tazarotene is a compound similar to vitamin A. It helps the skin to renew itself more quickly and may improve the appearance and texture of skin. The brand of Tazarotene cream is used to reduce the appearance of fine wrinkles on the face, mottled light and dark skin patches on the face, and benign facial lentigies (non-cancerous freckles) in adults and adolescents who are at least 17 years old.

Hydroquinone, a hydroxyphenolic chemical compound, inhibits the conversion of dopa to melanin by inhibiting the tyrosinase enzyme. It may also function by interfering with the formation or degradation of melanosomes and by inhibiting the synthesis of DNA and RNA within melanocytes. Its chemical resemblance with certain melanin precursors (tyrosine and dihydroxyphenylalanine) explains its ability to be metabolized in melanocytes as well as its selective action on melanogenesis. Unlike the monobenzylether of hydroquinone, Hydroquinone is not metabolized to cytotoxic free radicals and, therefore, is not a melanocidal agent. The depigmented effects are limited to the site of application and are usually reversible, although some investigators claim that Hydroquinone can cause permanent or vitiligo-like hypopigmentation, especially in darker skin types. The adjunctive use of hydroquinone can enhance the efficacy of Tazarotene in reducing dyspigmentation associated with photodamage.

Liposomes are lipid vesicles and one of the most suitable drug delivery systems to deliver the drug to the target organ and minimize the distribution of the drug to non-target tissues. Liposomes can enhance drug absorption achieved through their ability to come into intimate contact with the adjacent surfaces. The aim of the study the gel formulation of Liposomes
contain Tazarotene in combination with hydroquinone effectively maintains concentrations of active agents to the deep layers of the skin and/or the systemic circulation.

The drug Tazarotene and Hydroquinone were received as gift sample from Bioplus Life Sciences Bangalore. Their identity and purity were checked using melting point, UV and IR spectral studies. The absorption Maxima of Tazarotene and hydroquinone in PBS were determined by scanning on Shimadzu UV-1800 UV/visible double beam spectrophotometer. On scanning the solution of Tazarotene and hydroquinone in the range of 200-400 nm, absorption maxima were observed at 351 nm for Tazarotene and at 288 nm for Hydroquinone in buffer pH 7.4 and ph 6.8. The standard curves of drug in buffers ph 7.4 and ph 6.8 were prepared. The correlation coefficients obtained were 0.999 and 0.998 in each case, which is an indication of significant linear relationship between absorbance and concentration in this media.

The solubility of drugs was determined in various solvents at room temperature. The solubility of Tazarotene was found as in distilled water 0.00075 mg/ml, 0.1 N Hydrochloric acid 16.36 mg/ml, ethanol 17.57 mg/ml, methanol 26.26 mg/ml, Chloroform 18.56 mg/ml, acetone 15.58 mg/ml, sulphoxide 35.15 mg/ml, PBS (pH 7.4) 8.12 mg/ml, (pH 6.8) 9.25 mg/ml and for Hydroquinone solubility was found in distilled water 11.26 mg/ml, 0.1 N Hydrochloric acid 22.56 mg/ml, ethanol 25.65 mg/ml, methanol 31.25 mg/ml, Chloroform 5.26 mg/ml, acetone 8.56 mg/ml, sulphoxide 15.56 mg/ml and PBS (pH 7.4) 22.15 and (Ph 6.8) 25.37 mg/ml.

The Partition Coefficients of Tazarotene and Hydroquinone in octanol: Water systems were found to be 1.21 ± 0.001 and 0.85 ± 0.002 respectively.

Data of solubility and partition coefficient indicated the hydrophobic nature of Tazarotene and hydrophilic nature for hydroquinone. In order to estimate Tazarotene and hydroquinone in the experimental protocols, calibration curves of both drugs were prepared in PBS (pH 7.4) and (pH 6.8).

The absorption data for both drugs were found to follow beer Lambert’s law in the selected concentration range 1-5 μg/ml and 10-50 μg/ml for Tazarotene and Hydroquinone respectively. The method is also convenient, quick, less expensive, fairly sensitive and reproducible.
Significant development has been reported on combination of the liposome-based technology with temporary depot polymeric-based technology in sustaining drug release over prolonged periods of time. Integration of the more advanced types of liposome-based technologies such as targeted- or stimuli-sensitive liposomes in this system can enhance therapeutic efficacy. In addition, targeted liposome formulations, with targeted moieties such as antibodies, peptide, glycoprotein, polysaccharide, growth factors, carbohydrate, and receptors may increase liposomal drug accumulation in the tissues/cells via over expressed receptors, antigen, and unregulated selections.

In this study an attempt has been made to formulate a gel for dermal therapy of Tazarotene and Hydroquinone. The gel formulated consists of the Tazarotene loaded liposomes which are then included in the gel containing hydroquinone. Tazarotene liposomes were prepared by lipid film hydration method with required modifications after optimizing formulation variables.

The gel formulation of liposomes contain Tazarotene in combination with hydroquinone effectively maintains concentration of active agents to the deepest layer of the skin.

Cholesterol was included to improve bilayers characteristics of liposomes, increasing the micro viscosity of the bilayers which in turn results in the stabilization of the membrane and increase in the rigidity of the vesicles. Many methods for preparations of liposomes are described in literature. Most commonly used is the film hydration method and hence the same was utilised.

The aim of the study was to statistically optimize the vesicular formulation (liposomes) for enhanced skin delivery of a model drug Tazarotene in combination with gel containing Hydroquinone. Tazarotene was potent candidate, the active form of the drug Tazarotene acid is highly bound to plasma proteins (>99%), these factors promote researchers to choose this drug for the formulation of controlled release which maintain adequate concentration inside body to treat acne. Hydroquinone exploits the antioxidant properties when it is given in combination with Tazarotene liposomal gel, it maintain adequate concentration of drug in blood and help for effective management of acne.

But stratum corneum forms the most formidable barrier for the penetration of drug through skin. To overcome the stratum corneum barrier the use of lipid vesicles like liposomes in delivery system has attracted the attention which is increasing in recent years.
The Liposomal encapsulation of Tazarotene was found to increase the skin residence time leading to a faster healing of external lesions and to a reduction of side effects and duration of therapy. 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 partitioning into the skin. Liposomes have been reported to invade the skin intact and go deep enough to be absorbed by the systemic circulation.

For the present study liposomes were prepared by lipid film hydration method (as reportedly) by using rotary vacuum evaporator with modifications.

Regular 2³factorial designs were employed for screening of significant formulation and process variables involved in the development of liposomes. Description of high and low levels of various variables screened for their influence in the development of liposomes. Eight liposome formulations were designed using three factors - lecithin, cholesterol, rotation speed and two levels as high & low. Drug (Taza) lecithin, cholesterol and rotation speed ratio were altered where drug Tazarotene was constant (0.1%) used. Vesicle size and drug entrapment efficiency were studied. Chloroform: Methanol (2:1) mixture of different ratio and lecithin, cholesterol evaporated under vacuum at 40º±0.5ºC to form a lipid film on the wall of a round bottom flask. The resulting lipid film was then hydrated with PBS (pH 7.4) for 2 hours at 37º±0.5ºC. The preparation was sonicated at 4ºC in three cycles of 30 seconds & the formulation was homogenized at 1500 psi Pressure in 3 cycles using high pressure homogenizer to get Tazarotene loaded liposomes. The prepared liposomes characterized by vesicle size, zeta potential & entrapment efficiency.

Results showed that in formulation TL6 which contain lower level of Lecithin Cholesterol and rotation speed showed decrease in vesicle size found to be 180.4nm favorable Zeta potential was -37.5 mv and increase in entrapment efficiency found to be 69.10±1.52%.

On the basis of results obtained from the study TL6 was selected as optimized formulation.

Transmission electron microscopy was used to study the surface morphology of the optimized liposomal formulation (TL6). For TEM a drop of the sample was placed on a carbon coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and sample
was viewed on a transmission electron microscopy (TEM Hitachi H-7500 Tokyo, Japan) and photomicrograph was taken at suitable magnification. Photomicrograph shown in figure 7.12.

The TEM characterization revealed that the liposomes are small, spherical vesicles, however, some variation in size distribution was observed in the TEM image which might be attributed to an uncontrolled charge neutralization process involved between oppositely charged chains occurring during the formulation of liposome at specific pH. TEM revealed that liposomes have mean size of 100-500 nm.

After that three different concentration range .5%, 1% & 2% Carbopol gel base (LF1, LF2, and LF3) respectively were prepared.

Carbopol 934 (0.5g) was weighed and dispersed in 100ml of 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 the gel for maintaining consistency and Preservatives (methyl Paraben and Propyl Paraben) also added into the gel. Similarly 1% & 2% Carbopol gels were prepared. Liposomal gel was prepared by optimized liposome formulation (weight equivalent to 10 mg) dissolved in 10 ml of ethanol and centrifuged at 6000 rpm for 20 minutes to remove the unentrapped drug. The supernatant was decanted & sediment was incorporated into the gel vehicle.

Incorporation of the Tazarotene loaded liposomes (equivalent to 0.1%), and direct incorporation of hydroquinone (4%) into gel was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer, Vasai India) for 10 minutes. The optimized liposome formulation TL6 was incorporated into three different gel concentration 0.5%, 1% and 2% w/w.

Liposomal gels were evaluated by means of PH, Spreadability, viscosity and in-vitro drug release studies. PH was determined using digital Ph meter. The pH values of the prepared liposomal gels were within acceptable limits of 7.0-7.2 for LF1 (7.2±0.024) LF2 (7.0±0.035) LF3 (7.2±0.045). The Spreadability was measured on the basis of slip and drag characteristics of the gels and was in the range of 10.45 – 12.32 gms/cm sec. The Spreadability of LF1, LF2, and LF3 was found to be 10.45± 0.075, 12.32±0.042 and 11.75±0.049 respectively.

The viscosity of gels was determined by using a Brookfield viscometer DV-II model. The temperature which alters the viscosity was maintained at 25 °C because the increase of temperature decreases the viscosity of gels and vice versa. Viscosity for LF1, LF2 and LF3 found to be 1870±25 cps, 1895±33 cps and 1875±21 cps respectively.
In-vitro diffusion study of the liposomal gels (LF1, LF2, and LF3) was performed using modified Franz diffusion cell with dialysis membrane in phosphate buffers pH 6.8 for a period of 12 hours for Tazarotene and period of 30 minutes for Hydroquinone.

In order to determine the exact mechanism of drug release from liposomal gel the in vitro release data were fitted to Korsmeyer-Peppas equation and the ‘n’ values were calculated. ‘n’ values were found to be in the range of 0.5<n<1.0, which suggest that the drug release mechanism from the gel followed non-Fickian diffusion mechanism (Anomalous transport). Liposomal gel released drug in controlled release manner in 12 hours but in case of marketed formulation there is no controlled release of drug from gel.

In-vitro drug release data for LF, LF2 and LF3 upto 30 minutes for Hydroquinone was found to be 98.75±0.28, 98.89±0.56 and 95.45±0.36 respectively and in vitro drug release data for LF1, LF2, and LF3 upto12 hours for Tazarotene was found to be 97.08±0.11, 98.120±0.45 and 78.890±0.25 respectively.

Percentage cumulative drug (Taza) released after 12 hours from LF1, LF2 and LF3 liposomal gel formulation was 97.08±0.11, 98.120±0.45 and 78.89±0.25 respectively. % cumulative drug (Hydroquinone) released after 30 minutes from LF1, LF2 and LF3 liposomal gel formulation was 98.75±28, 98.89±56, 95.45±36 respectively.

The drug release data was screened for different models to ascertain as to which the data fits in the best and in order to know the type of release of the drug from the delivery system. Based on the results and the regression coefficient (maximum 0.976 for Higuchi model for LF2 formulation) it was found that the drug release from the system followed a non-fickian curve and also represent higuchi model which shows that the drug release through the delivery system was controlled and the drug released through matrix which is due to the gel system. The same also can be attributed to the liposomal vesicular system which delivers the drug in a controlled manner.

The release of the drug 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. Result showed that LF2 was optimized formulation.

The stability study of optimized formulation was performed at different temperature 4±1°C and 25±1°C for a period of four weeks. There was no significant variation found in physical
appearance but increases vesicle size and decreases % drug content of the liposomal gel LF2 as the temperature increases. After analysis it was observed that degradation rate of drug was maximum at 25±1ª and minimum at 4.0±1ª. Stability studies performed for Liposomal gel indicates the prepared formulation have more stability at freezing temperature than that of room temperature. Drug molecules could be successfully entrapped in liposomes and gel with reasonable drug loading. Hence from results obtained it can be concluded that liposomal gel containing drugs has potential in transdermal delivery. No visible changes in the appearance of the gel formulation were observed at the end of the storage period.

For evaluating the antibacterial activity the strain of Propionibacterium acnes obtained from National Centre for Cell Science, Pune Maharashtra, India. The lyophilized cultures of bacterial strain upon culturing in nutrient broth for 24 hours at 37±5ª in an incubator resulted into turbid suspension of activated live microbial cell ready to be used for microbiological study. From the broth of respective revived cultures of micro organism loop full of inoculums is taken and streaked on to the nutrient agar medium and incubated again at same culture conditions and duration that yielded the pure culture colonies on the surface of the agar culture that are successfully stored in refrigerated conditions at 4ª as stock culture to be used for further experimentation.

Sensitivity of microorganism towards the antibacterial liposomal gel formulation and marketed Tazarotene gel was studied at the concentration of 25, 50, and 100 µg/ml using well diffusion method and zone of inhibition were seen. Liposomal gel at all the concentration (25, 50 and 100 µg/ml) used in study for comparison.

In present work, liposomal and marketed gels showed antibacterial activity against Propionibacterium acnes with maximum zone of inhibition lying in the range of 18 to 26 mm (figure 7.29)

On comparison of formulated liposomal gel with marketed gel of Tazarotene, liposomal gel showed greater percentage of inhibition of bacterial infection against Propionibacterium acnes.

In present in-vivo anti acne activity Clindamycin was selected as a standard drug and showed the effect of Clindamycin, Formulation- I (marketed Tazarotene gel) and formulation II(liposomal gel LF2) on acne and mean thickness compared to the normal. Results showed in table 7.34 and figure 7.31-7.32.
It was observed that formulation-I (marketed Tazarotene gel) and formulation-II (liposomal gel LF2) showed a significant reduction in the acne without necrosis as compared with the standard Clindamycin.

Various antibiotics like tetracycline, Clindamycin & erythromycin etc. and other drugs like benzoyl peroxide are used for acne treatment. The various drawbacks for synthetic drugs are different side effects and resistant developed towards these drugs. Formulation therapy is required to overcome the above drawbacks & treat the acne.

In conclusion it can be proposed that the liposomal gel have proved to be efficient carrier for the transdermal drug delivery of drug molecules. Liposomes are lipid vesicles and one of the most suitable drug delivery system to deliver the drug to the target site and minimize the distribution of the drug to non-target tissue. Liposomes can enhance drug absorption achieved through their ability to come into intimate contact with the adjacent surfaces. The developed liposomal gel based formulation can prove to be very instrumental in the efficient cure of the acne. The formulation can be scaled up for industrial purpose since the same is very simple to prepare. Moreover since the formulation is a combination of the drugs and hence positive results with rapid cure can be achieved. However clinical correlation and more evident research may be needed for the same to be utilized for human use in cure of acne.