Research Article

Formulation development and evaluation of Transferosomal drug delivery for effective treatment of acne

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Abstract

Objective: The objective of the present work was to formulation, development and evaluation of transferosomal drug delivery for effective treatment of acne. **Materials and Methods:** Soya phosphatidyl choline, sodium cholate, Tazarotene, ethanol and all chemicals and reagents used were of analytical grade. **Results:** Different formulation (F-1 to F-8) of transfersomes were prepared and evaluated for vesicle size and entrapment efficiency. The vesicle size of all transfersomes varied between 155.2 and 262.1nm where as entrapment efficiency was found between 60.12 to 75.65%. Results showed that in formulation F4 which contain smallest vesicle size and increase in entrapment efficiency, Formulation F4 selected as optimized formulation and further incorporated into gel base (TF1, TF2, TF4) and evaluated for Drug content, pH, Spreadibility, Viscosity measurements and *In-vitro* drug release study. Transfersomes gel released drug in controlled release manner in 12 hour but in case of marketed formulation there is no controlled release of drug from gel. **Conclusion:** The developed of Tazarotene as transfersomal gel has the ability to overcome the barrier properties of the skin and increase the drug release

Keyword: Acne vulgaris, transfersomes, tazarotene, development, evaluation, topical

Introduction

The term Transfersome and the underlying version had been delivered in 1991 by way of Gregor Cevc. Since then, large quantity of research is going on international on these elastic vesicles under various titles like flexible vesicles, phytosomes, ethosomes, microsponge and nano-formulations etc (Vikas et al 2012, Shukla et al 2016, Garg et al., 2018). In broadest feel, a Transfersome is a noticeably adaptable, pressure responsive and multifaceted mixture. Its favored form is an ultra deformable vesicle owning an aqueous core surrounded by using the complex lipid bilayer. Transfersome is a term registered as a hallmark via the German corporation IDEA AG, and utilized by it to consult its proprietary drug transport era. The name way “wearing frame”, and is copied from the Latin phrase 'transferre', which means 'to carry throughout', and the Greek phrase 'soma', for a 'frame'. A Transfersome service is an artificial vesicle and resembles the herbal cell vesicle. Thus it's far appropriate for targeted and controlled drug shipping (Prajapati et al., 2011). Transfersomes are vesicles, which are self-optimized aggregates with extremely-bendy membrane. These vesicular transfersomes are greater elastic than the standard liposomes and as a result nicely perfect for the skin penetration (Gaur et al., 2003).

There are numerous times in which the maximum suitable drug intake techniques, like oral course, have been now not viable and opportunity routes had to be sought. Though, intravenous management of the medicament avoids many of those shortfalls (inclusive of gastrointestinal and hepatic metabolism), its invasive and apprehensive nature (especially for chronic management) has advocated the look for opportunity techniques. Transdermal Topical drug delivery gives numerous wonderful blessings together with relatively large and effectively to be had surface area for absorption, ease of utility and termination of therapy.

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- Transfersomes release the drug in a continued manner for a prolonged time frame at a predetermined charge.
- Transfersomes can distort and skip through narrow constriction (from 5-10 instances less than their own diameter) without measurable loss.
- Transfersomes can act as a service for low and high molecular weight tablets.
- Transfersomes have high entrapment performance.
- Transfersomes are used for each, pertinent and systemic delivery of medicine
- They shield the encapsulated drug from metabolic degradation (Yoshioka et al., 1994, Nagasamy et al., 2014, Sayali et al., 2015).

Materials and methods

Tazarotene, Lecithins, Soya phosphotidyl choline and all the other chemical and reagents were analytical grade used.

Preformulation

Preformulation study is the first step in the rational development of dosage form of a drug substance. It can be defined as an investigation of physical and chemical properties a drug substance alone and when combined with excipients. Preformulation studies include studies of:

(i) The physiochemical properties of drug, and an assessment of their relevance to the final formulation. (ii) The chemical and physical stability of drug. (iii) Chemical /physical compatibility of the active with potential excipients. These studies give clues as to how to achieve the desired performance of the finished products.

Physiochemical Properties of Tazarotene

Physical evaluation

It refers to the evaluation by sensory characters-taste, appearance, odor, feel of the drug, etc.

Melting point

It is one of the parameters to judge the purity of drugs. In case of pure chemicals, melting points are very sharp and constant. Since the drugs contain the mixed chemicals, they are described with certain range of melting point.

A small quantity of powder was placed into a fusion tube. That tube was placed in the melting point determining apparatus (Chemline) containing castor oil. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

Determination of pH (1%w/v solution in water)

About 100mg of the Powder was taken and dissolved in 100ml of distilled water with sonication and filtered. The pH of the filtrate was checked with standard glass electrode.

Identification Test

Infra- red spectrum is an important record which gives sufficient information about the structure of a compound. This technique provides a spectrum containing a large number of absorption band from which a wealth of information can be derived about the structure of an organic compound. The region from 0.8 μ to 2.5 μ is called Near Infra-red and that from 15 μ to 200 μ is called Far infra-red region Identification of Tazarotene was done by FTIR Spectroscopy with respect to marker compound. Tazarotene was obtained as Light yellow crystalline powder. It was identified from the result of IR spectrum as per specification and sample of pure Tazarotene. The IR spectrum of sample drug shows the peak values which are characteristics of the drug.

Loss on drying

Loss on drying is directly measured by IR moisture balance. Firstly calibrated the instrument by knob then taken 5.000 gm sample (powder) and set the temp at 100°C to 105°C for 15 minutes and constant reading set the knob and check % moisture (Jain et al., 2001).

Moisture content determination

Karl Fischer volumetry is used for samples with high water content, i.e. 1-100 mg per sample. An iodine-containing solution serves as titrating agent. The water content of the sample is calculated using titration volume and titer of the titrating agent. One-component reagents conveniently contain all reactants (Iodine, sulfur dioxide and a base) dissolved in a suitable alcohol in one solution, whereas two-component reagents contain all necessary reactants separated in two different solutions to enhance the rapidity of the Karl Fischer reaction and the titer stability of the titrating agent.

Karl Fischer coulometry is a micro-method and is particularly suitable for samples with low water content, from 10 μg up to 10 mg. Here, the required iodine is generated electrochemically in the titration vessel by anodic oxidation from iodide contained in the coulometric reagents. The amount of consumed electric charge is used to calculate the consumption of iodine and therefore the amount of water in the sample.

Determination of λmax of Tazarotene

Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.4 pH buffer solution in 10 ml of volumetric flask (Kumar et al 2018). The resulted solution 1000μg/ml and from this solution 1 ml pipette out and transfer into 10 ml

volumetric flask and volume make up with 7.4 pH buffer solution prepare suitable dilution to make it to a concentration range of 5-25 µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). The spectrum peak point graph of absorbance of Tazarotene versus wave length was shown in figure.

Formulation development of Tazarotene loaded transfersomes

Soya-phosphatidylcholine, Span 80 and Tazarotene were dissolved alcohol. Then solution was put in a round bottom flask. These were then dissolved by shaking. Thin film was then formed by keeping it in the rotator vacuum evaporator at 40^0C. Final traces of solvent are removed under vacuum. The deposited lipid film is hydrated with the appropriate buffer by rotation at 60 rpm for 1 hour at room temperature. The resulting vesicles are swollen for 2 hours at room temperature. The multilamellar lipid vesicles (MLV) are then sonicated at room temperature. This thin film was then hydrated by phosphate buffer saline to get the transferosome (Jain et al., 2007).

Characterization of Tazarotene loaded transfersomes

Vesicle size determination: Vesicle size was determined using the particle size analyzer (Malvern Master Sizer, Malvern Instruments Ltd., Malvern, UK) (Kumar et al., 2004).

Entrapment efficiency

Tazarotene was estimated in transfersomes by ultra centrifugation method and in transfersomes by protamine aggregation method followed by ultra centrifugation. The total volume of the transfersomes suspension was measured and 2 ml of this formulation was transferred to 10 ml centrifuge tube. The suspension was diluted with distilled water up to 5 ml and centrifuged at 2000 rpm for 20 minutes to separate out un-dissolved drug in the formulation. Transfersomes were separated by ultra centrifugation at 20,000 rpm for 30 minutes. Supernatant and sediment was recovered and their volume was measured. Sediment was diluted with distilled water up to 5ml. The unentrapped drug contents were analyzed by estimating drug in supernatant by spectroscopic method (Kumar et al., 2007; Patel et al., 2009).

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 min 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).

Preparation of transfersomes gels

1g of transfersomes 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 (Preeti et al., 2014). The incorporation of the transfersomes into gels was achieved by slow mechanical mixing at 25 rpm 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 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.

Spread-ability

A modified apparatus suggested was used for determining spread-ability. The spread-ability 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 lower one was fixed to a wooden plate and the upper one was attached by a hook to a balance. The spread ability was determined by using the formula: $S = \underline{mxl}/t$, where S, is spread-ability, m is weight in the pan tied to upper 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 spread-ability 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.

(a) Selection of spindle: The best method for the selection of spindle was based on trial and error startied from T91 to T95 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.

(b) **Spindle Immersion**

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

(c) **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.

Drug content

1 gm. of the prepared gel was mixed with 100 ml. of ethyl alcohol. Aliquots of different concentrations were prepared by suitable dilutions after filtering the stock solution and the absorbance was measured at 296 nm. Drug content was calculated by linear regression analysis of the calibration curve (Patel and Patel, 2013).

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. Transfersomes gel equivalent to 500 mg of Tazarotene was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (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 (Rizwan et al., 2004). The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage forms characteristics are used.

Stability studies

Optimized formulations of transfersomes gel were subjected to accelerated stability testing under storage condition at 4 ± 1^0C and at room temperature (28 ± 1^0C). Both formulations were stored in screw capped, amber colored small glass bottles at 4 ± 1^0C and 28 ± 1^0C. Analyses of the samples were characterized for vesicle size and drug content after a period of 7, 14, 21 and 28 days (Thakur et al., 2018).

Antibacterial activity (Malakar et al., 2012)

Pathogenic bacteria used

The pathogenic bacteria used in the current study *Acne vulgarise* was obtained from Microbial type culture collection, Institute of microbial technology, Chandigarh, Punjab, India.

Media preparation: Composition of nutrient agar media

Agar	:	1.5 gms.
Beef extract	:	0.3 gms.
Peptone	:	0.5 gms.
Sodium chloride	:	0.55 gms.
Distilled water to make:		100 ml.
pH	:	7

This agar was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients were transferred to a conical flask containing required quantity of distilled water and were heated to dissolve them completely.

Sterilization culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch2 (121°C) for 15 minutes.

Preparation of plates

After sterilization, the molten agar in flask was immediately poured (20 ml/ plate) into sterile Petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37°C overnight to check the sterility of plates. The plates were dried at 50o±0.5°C for 30 minutes before use.

Anti-acne activity studies

Broth cultures of the pure culture isolates of microorganisms *Acne vulgarise* which are sensitive towards the 100 mg/ml concentration of gel formulation used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37±0.5 °C for 48hours. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture. The well diffusion method was used to determine the antibacterial activity of gel formulation using standard procedure. There were 3 concentration used which are 10, 20 and 30 μg/ml for gel formulation for antibiogram studies. Undiluted over night broth cultures should never be used as an inoculums. Routine direct application of discs to plates seeded with clinical material is not recommended because of problems with inoculam control and mixed cultures. The plates were incubated at 37±0.5 °C for 48 hour and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

Results and discussion

Physiochemical Properties of Tazarotene

Physiochemical property of Tazarotene was to be found in light yellow colour powder, melting point range 95-96^{0}C and pH 6.96, slightly acidic. Show that this drug fit for the

development of topical formulation.

Identification Test

Identification of Tazarotene was identified by using FTIR, The peaks of FTIR spectra showed all their characteristics peaks, and where also studied drug interaction between drug and excipient mixture. The spectra of FTIR represented information about any interaction which is shown in figure 1.

Loss on drying

Loss of drying test of drug sample was performed in thrice and found to be average reading 1.72±0.07.

Table 1. Determination of moisture content

S. No.	Initial weight	Final weight after 15 minutes	% loss of drying	Avg. % loss of drying
1.	5gm	4.92 gm	1.67 %	1.72±0.07
2.	5gm	4.91 gm	1.82 %	
3.	5gm	4.92 gm	1.67 %	

SP=span 80, PC=phosphatidylcholine

Table 2. Formulation code and variable used in preparation of transferosome

S. No.	Formulation code	PC:S (mg)	Drug (mg)
1	TF1	95:05	100
2	TF2	90:10	100
3	TF3	85:15	100
4	TF4	80:20	100
5	TF5	8:20	100
6	TF6	85:15	100
7	TF7	90:10	100
8	TF8	95:05	100

Moisture content determination

Moisture content of drug sample was performed by using KF instrument and moisture content where found to be 0.113 (Table 1).

Determination of λ max of Tazarotene

The calibration curve was prepared by using UV spectroscopy at pH 7.4 in phosphate buffer of range of 10-

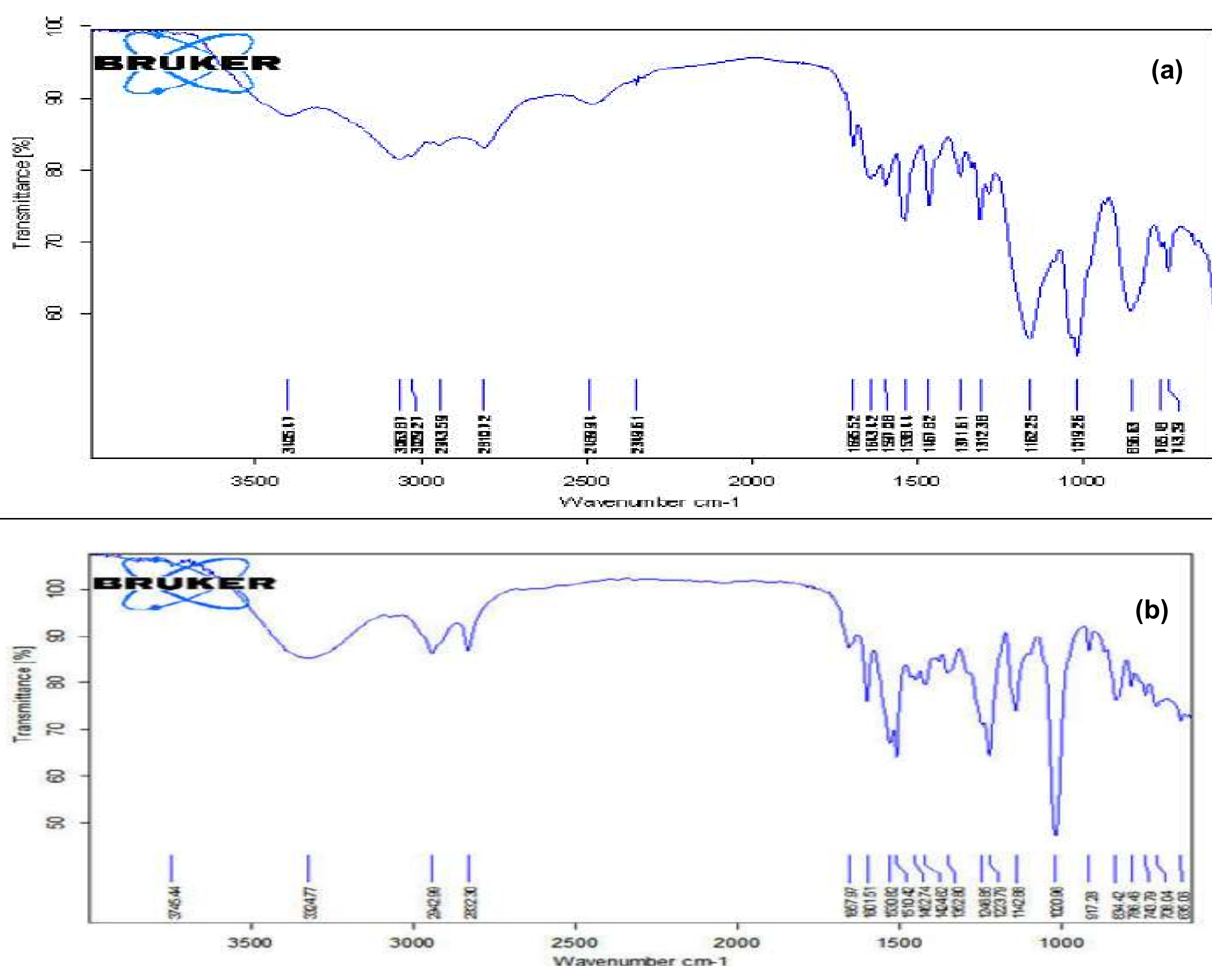

Figure 1. FT-IR Spectrum of (a) Tazarotene; (b)Tazarotene + All Excipients (Narayana Raju, 2009)

30µg/ml concentration. The λ max of Tazarotene where found to be 351nm. The linear regression analysis was calculated. The results are as follow for standard curve, slope = 0.030, intercept = 0.003 and the correlation coefficient (r2) = 0.999. The UV spectra and calibration curve are shown in figure 2.

Formulation development

In the formulation of transferosomes where used SP=span 80, PC=phosphatidylcholine. The formulation code and ratio of polymers and drug contents are shown in table 2.

Characterization of Tazarotene loaded transfersomes

Results were includes the value of vesicle size, and entrapment efficiency. The vesicle size of all transfersomes varied between 155.2 and 262.1nm where as entrapment efficiency was found between 60.12 to 75.65%.

Results showed that in formulation TF4 which contain smallest vesicle size and increase in entrapment efficiency, Formulation TF4 selected as optimized formulation for further evaluation.

Vesicle size determination: Vesicle size was determined using the particle size analyzer (Malvern Master Sizer, Malvern Instruments Ltd., Malvern, UK) (Kumar et al., 2004). Evaluation of tazarotene loaded transferosomes is shown in table 3.

Table 3. Evaluations of transfersomes for Vesicle size and Entrapment efficiency

Formulation	Vesicle Size (nm)	Entrapment efficiency (%)
TF1	155.2	60.12
TF2	160.2	65.58
TF3	220.3	78.98
TF4	123.1	75.65
TF5	155.6	63.45
TF6	166.5	70.23
TF7	245.6	69.98
TF8	262.1	70.45

Entrapment efficiency

The vesicle size of all transfersomes varied between 155.2 to 262.1nm where as entrapment efficiency was found between 60.12 to 75.65%. Results showed that in formulation F4 which contain smallest vesicle size and

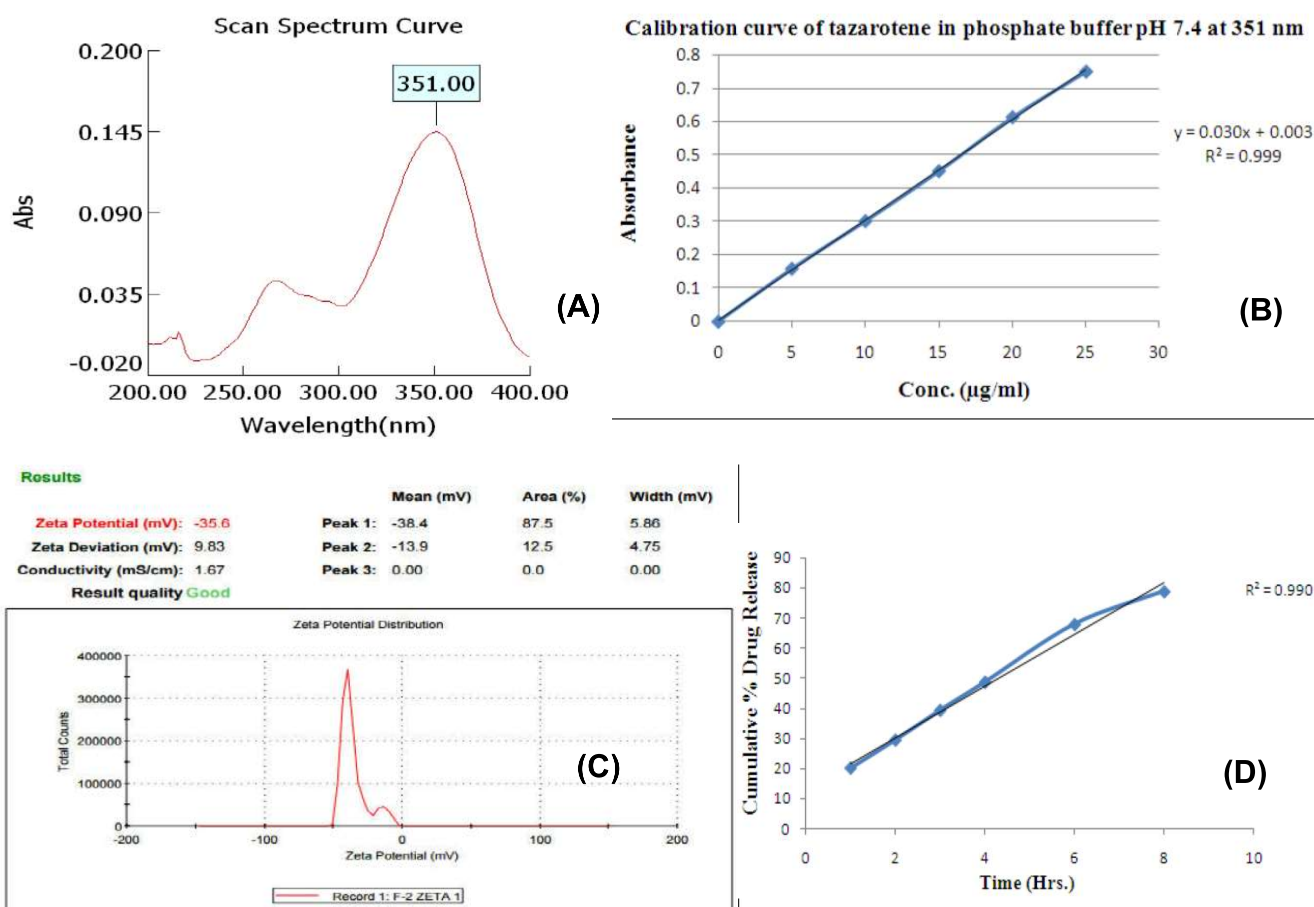

Figure 2. (A) λmax of Tazarotene in phosphate buffer pH 7.4; **(B)** Calibration curve of tazarotene in phosphate buffer pH 7.4 at 351 nm; **(C)** Zeta potential of Optimized transfersomes formulation; **(D)** Cumulative %t drug released Vs Time

Table 4. Results of transfersomes gel formulations TF4

S.N.	Drug content (%)	pH	Spread-ability (Gm.cm/sec.)	Viscosity (cps)
1	95.65	6.9	18.98	4056
2	97.89	7.1	15.65	4089
3	92.23	7.5	13.25	4156

Table 5. *In-vitro* drug release data of transfersomes gel formulation TF4

Time (h)	Square Root of Time(h)1/2	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	1	0	20.25	1.306	79.75	1.902
2	1.414	0.301	29.56	1.471	70.44	1.848
3	1.732	0.477	39.45	1.596	60.55	1.782
4	2	0.602	48.78	1.688	51.22	1.709
6	2.449	0.778	67.98	1.832	32.02	1.505
8	2.828	0.903	88.89	1.949	11.11	1.046

*Average of three readings

increase in entrapment efficiency, Formulation F4 selected as optimized formulation for further evaluation. Optimized formulation (TF4) showed vesicle size (nm), entrapment efficiency and zeta potential as 123.1, 75.65 and -38.4, respectively.

Transmission Electron Microscopy (TEM)

The optimized formulation TF4 of transferosomes size was identified by using TEM and was found to be vesicle size (nm) 123.1, Entrapment efficiency 75.65 and zeta potential -38.4. The spectra of zeta potential are shown in figure 2.

Evaluation of Gels

Optimized formulation of Tazarotene loaded transfersomes gel where evaluated and performed the entire test in thrice. The results were found to be in range of drug content 92.23-97.89, pH 6.9-7.5, spread-ability 13.25-18.98 and viscosity 4056-4156. The results are shown in table 5 and 6.

***In-vitro* diffusion study**

Transfersomes gel (TF1, TF2, and TF4) was performed using modified Franz diffusion cell with dialysis membrane in phosphate buffer pH 7.4 for a period of 8 hours. The data obtained from diffusion studies are summarized in table. The release rate of Tazarotene from transfersomes formulation over dialysis membrane was significantly higher than its transport across skin, indicating the barrier properties of skin for drugs. The in vitro release data were fitted into different kinetic models viz Zero-order value 0.990, First order 0.989, Higuchi model 0.989 and Korsmeyer Peppas 0.994 respectively. Zero-order plots, First order plots, Higuchi plots and Korsmeyer Peppas lots are shown in figure 2 and 3. The zero-order plots were found to be 0.990 fairly linear. In order to determine the exact mechanism of drug release from marketed clindamycin 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 suggests that the drug release mechanism from the gel followed non-Fickian diffusion mechanism (Anamolous transport). The regression analysis data of transfersomes gel formulation are shown in table 6. Transfersomes gel released drug in controlled release manner in 12 hour but in case of marketed formulation there is no controlled release of drug from gel. The release of the drug from transfersomes gel was found to follow the order: TF2> TF3> TF4.

Table 6. Regression analysis data of transfersomes gel formulation

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
	R^2	R^2	R^2	R^2
TF4	0.990	0.989	0.989	0.994

Stability studies

Stability studies for optimized formulations were carried out at

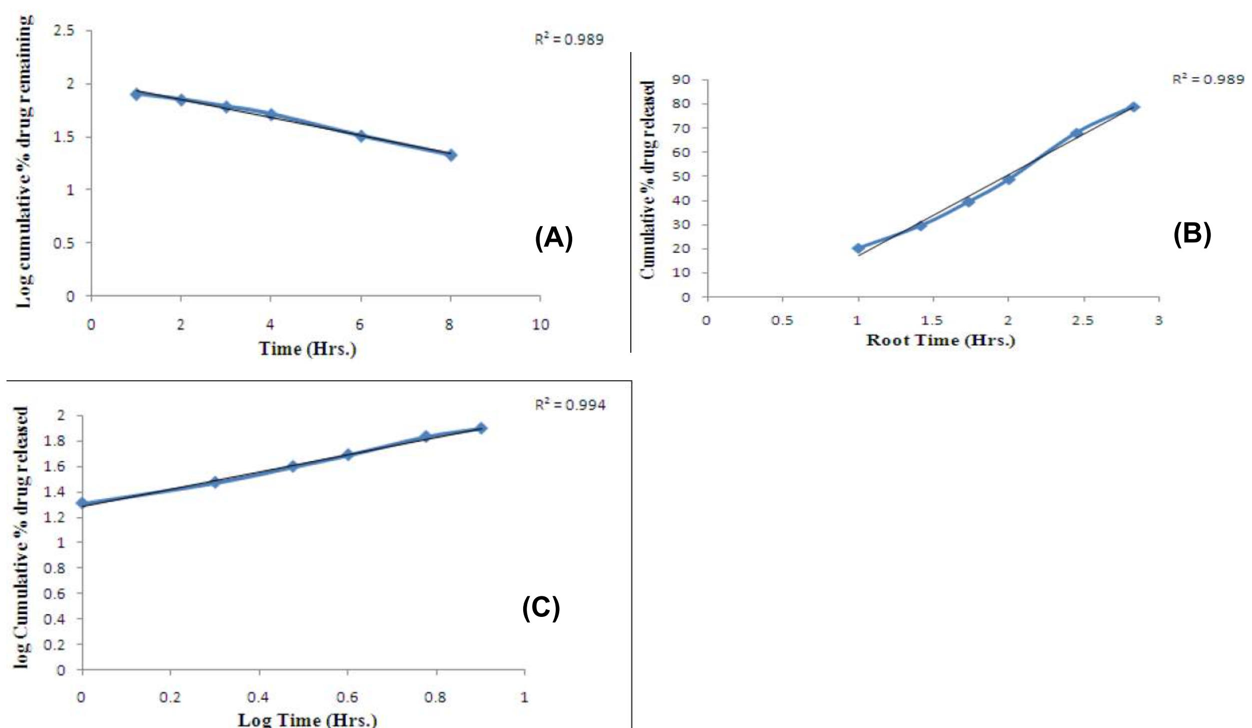

Figure 2. (A) λmax of Tazarotene in phosphate buffer pH 7.4; **(B)** Calibration curve of tazarotene in phosphate buffer pH 7.4 at 351 nm; **(C)** Zeta potential of Optimized transfersomes formulation; **(D)** Cumulative %t drug released Vs Time

40.0 ± 0.5°C and 28 ± 0.5°C for a period of four weeks. There was no significant variation found in appearance, average particle size and % drug content of the transfersomes gel (Thakur et al., 2018).

Antibacterial activity (Malakar et al., 2012)

The present investigation in this research work, the antimicrobial activity of optimized gel formulation was evaluated against *Acne vulgarise* under present study.

In vitro anti-acne activity results for Tazarotene transferosomal gel TF4 formulation, against fungal strains of *Acne vulgarise* (causative organism for fungal acne, face fungus, *Acne vulgarise* etc.) were observed. Antimicrobial activity of Tzarotene against *Acne vulgarise* in the term of Zone of inhibition as 35±0.15, 29±0.13 and 25±0.19, at 30μg/ml, 20μg/ml and 10μg/ml.

Conclusion

Transfersomes systems are vesicular systems composed mainly of phospholipids, ethanol, and surfactant. Unlike classic liposomes that are known mainly to deliver drugs to the outer layers of skin, transfersomes were shown to enhance permeation through the stratum corneum barrier. Transfersomes are shown to entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic or amphiphilic. Tazarotene is a third-generation prescription topical retinoid sold as a cream, gel, or foam. Tazarotene is a member of the acetylenic class of retinoids. This medication is approved for treatment of psoriasis, acne, and sun damaged skin. It is commonly sold in two concentrations: 0.05% and 0.1%. Transfersomes offers a good opportunity for the non-invasive delivery of small, medium, and large-sized drug molecules. Studies will continue to further improve the skin delivery of drugs using lipid vesicles. Special emphasis seems to be given to the skin delivery of proteins and other macromolecules and for transcutaneous immunization. The near future also holds the emergence of new commercial transfersomes -based topical products. In this study an attempt has been made to formulate a supplement dermal therapy of Tazarotene. The Tazarotene encapsulation 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.

Conflicts of interest: Not declared.

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