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Design and Development of Surfactants Based Elastic Vesicular Drug Carrier System containing Antifungal Drug for Ophthalmic Disorder

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Abstract: The work depicts utility of a novel; surfactants based elastic vesicular drug carrier system (SEVs), for targeting topically applied drug(s) to the posterior segment of the eye. The system comprised span 20 and an edge activator (tween 80). Terbinafine hydrochloride is a synthetic allylamine antifungal with a low molecular weight (327.89 Da.). It is hydrophilic in nature and has high protein binding and a limiting solubilityis expected to show a poor transport across the cornea; hence no ocular formulations are available. It is used to treat ocular mycoses, the second most usual drive of blindness in developing countries. However, its administered poor patient compliance and limited use due to its variable half-life. Therefore, Terbinafine hydrochloride was comprised into a novel sorbitan (spans) based elastic (SEVs) vesicular system with intent to achieve a prolonged and better effect. It was found to be safe in terms of dermal and eye irritation/corrosion tests according to OECD guidelines. Safety was a crucial issue considering that the system is novel (Indian Patent Application 2390/DEL/2008; 1447/DEL/2010) and is completely surfactant based (spans plus edge activators) formulation.

INTRODUCTION

Eye is regarded as the main part of our soul. It is a unique organ of our body anatomically as well as physiologically (Zimmer and Kreuter 1995). There are retina, choroid, vitreous humour and optic nerve in the posterior part of the eye. They are mainly used to treat many diseases such as age-related macular degeneration, diabetic retinopathy, retinal arterial occlusion, glaucoma, macular edema, uveitis, postoperative inflammation, retinitis, retinal venous occlusions and proliferative vitreoretinopathy by drug delivery. It delivers easy to administer for local action and to non-invasive clinical approach. About 5% of dose instilled into the eyes targets the intraocular tissues that route becomes complicated by the effective removal processes of tear film of the eye which removes the foreign particles and also by the precorneal barrier which includes tear turnover, low permeability across the cornea by immediate drain away from cavity (Sahoo et al., 2008)

The main goal is to achieve desired pharmacological response by effective drug concentration on targeted site. There is poor bioavailability of ophthalmic drug delivery due to tear, transient residence time and corneal barrier. To treat ocular diseases with the drawback of poor ocular bioavailability of solution, suspensions and ointments for topical and localized approaches (Davis et al., 2004).

In this newly research, the drug delivery by elastic vesicles has gained more attention due to its prominent advantage in improving bioavailability and reducing dose frequency. These are the water-filled colloidal particles consists of amphiphilic (lipid and surfactants) molecules where hydrophilic drugs are closed in within the aqueous environment and lipophilic drugs are entrapped in the vesicle bilayer (Lang 1995). Vesicular drug delivery for localize and maintain drug concentration at its targeted site for action. The drug penetration rate depends on the physicochemical properties of the drug. This drug delivery system most preferred as liposomes and niosomes (Kaur et al., 2004).

Terbinafine hydrochloride is a white crystalline anti-fungal powder, with poor water solubility and tendency to cause a variety of side effects upon oral administration (nausea, vomiting, gastrointestinal disturbance, hepatitis, gynecomastia and adrenal cortex suppression). Also; it is readily absorbed from gastrointestinal tract and possible drug–drug interactions on oral administration. It is synthetic allylamine antifungal drug with molecular weight of 327.89 Da. (Akash and Bhanu 2013)

EXPERIMENTAL SECTION

Materials

Terbinafine hydrochloride was a kind gift from Dr. Reddy's Laboratories, India. Tween 80 and Span 20 were obtained from Loba chem Pvt. Ltd.,

Mumbai. All other reagents used in study were of analytical grades.

Preformulation Study of SEVs

The significant first step had purpose to study about to develop a collection of evidence for API and on the basis of this information developed new formulation. It was examined physical and chemical properties of API and also when it mixed with excipients. The following preformulation studies were performed in Terbinafine hydrochloride and other excipients (Ballie 1985)

1. Characterization of API
2. Drug - Excipient Compatibility Studies

1. Characterization of API

FTIR of API: FTIR (Fourier transform infrared) spectra were obtained on PerkinElmer FTIR Model- Spectrum RX 1 spectrometer for identification of API. The Spectra was recorded as a dispersion of the sample in Potassium Bromide in IR disk (2 mg sample in 200 mg KBr) with the scanning range of 400 to 4000 cm^{-1}.

Melting Point: Taking a pinch of Terbinafine hydrochloride into a capillary tube and closed at one end. It was then sited in an electrically operated micro controller based melting point apparatus - Chemi line (CL 725) and the temperature at which the drug melted was noted.

Absorbance: Take sample into one cuvette and another with blank sample. Then measure absorbance by UV spectrophotometer Lambda 25, PerkinElmer.

Calibration Curve of Terbinafine Hydrochloride (Indian Pharmacopoeia, 2010)

- Preparation of primary stock solution:

  10 mg accurately weighed of Terbinafine Hydrochloride and in 10 ml of volumetric flask dissolved it in small quantity of methanol and made up to 10 ml volume with methanol to produce stock solution which having a concentration of 1000 µg/ml.

- Preparation of secondary stock solution:

  From the primary stock solution, 1 ml of solution was taken in the 10 ml of volumetric flask and diluted up to 10 ml with methanol to produce secondary stock solution having concentration of 100 µg/ml.

- Preparation of diluted concentration:

  The concentration range of 2-20 µg/ml was prepared from diluting of the secondary stock solution with methanol individually. The absorbance of each dilutions was measured at λ_{max} 283.4 nm using methanol as a blank and standard curve was plotted between concentration (µg/ml) on X-axis and absorbance on Y-axis.

Solubility Studies: For solubility, equivalent amount of the drug was taken in different test tubes containing the different solvents (methanol, chloroform, 0.1N HCl and water). After the addition of each portion of solvent, This was done by dissolving excess drug in different flasks containing different solvents. The flasks were shaken thoroughly for 6 hours and kept aside for 24hours. The suspensions were filtered, diluted suitably and absorbance was measured at 283nm.

2. Drug - Excipient Compatibility Studies

Drug - Excipient Compatibility studies were carried out on optimized formulation. The samples were stored at 40 ± 2°C/75 ± 5% RH, at Room Temperature and in Freezer for duration of 1 month. The sample was collected weekly and tested by using PerkinElmer FTIR Model- Spectrum RX 1 spectrometer (Akash and Bhanu 2013).

Mixture Design using Design of Experiment

Mixture design was chosen for current formulation optimization study, in this design 2 factors were evaluated and experimental trials were performed at all 8 possible combinations which were showed by Design Expert. In the preliminary trial runs, Combination of Span 20 and tween 80 in concentering range 30-70 and 50-50. The 50-50 shows the optimized results, so these levels were selected and subjected to further optimization, span 20 & tween 80 concentration were selected as independent factors whereas particle size & % drug content were measured as responses. The polynomial equation can be used to draw conclusions by the magnitude of coefficient and the mathematical sign. The responses were analyzed for ANOVA using Design Expert version 8.7.1. A mathematical equation and model were generated for each response parameter and tested for significance. Response surface plots were generated for each response to study the behavior of the system. (United States Pharmacopoeia, 2000)

Preparation of SEVs

SEVs containing Span 20 and edge activator (EA) Tween 80 at a ratio of 50:50 (by weight) were prepared by ethanol injection method (Optimized batch). Terbinafine hydrochloride and span 20 were dissolved in ethanol. The solution so prepared is then transferred rapidly into the preheated tween 80

and vigorously stirred on the magnetic stirrer at high speed. The pH was adjusted to 7.4 using glacial acetic acid. Terbinafine hydrochloride was

Evaluation of Formulation

Morphology and Vesicle Size

SEVs were characterized by using Motic microscope for structural attributes such as lamellarity, uniformity of size, shape and physical stability characteristics i.e. aggregation and/or irregularity. The size of vesicles was analysed by Zetasizer (Malvern instrument, ZS 90).

Zeta Potential

Zeta potential of the vesicular dispersions was measured using the Malvern's zetasizer. The measurements were done at 25°C, and the electric field strength was around 23.2 V/cm. The count rate was 163.5 kcps and measurement position was 2 mm.

Total Drug Content

Isopropyl alcohol was chosen as a suitable solvent for disrupting the prepared vesicles. Aqueous dispersion (1 ml) was disrupted using sufficient quantity of isopropyl alcohol and the absorbance was recorded at 283.4 nm (Hillgren et al., 2002)

Differential Scanning Calorimetry (DSC)

The samples were scanned by using DSC for thermal analysis and the generated thermograms were observed for any significant shift of new peaks. It was performed with a DSC (Mettler Toledo, Star E Software). The calorimeter was calibrated for temperature and heat flow accuracy using the melting of pure indium (mp 156.6°C and ΔH of 25.45J gm−1). The temperature range was from 40 to 300°C with a heating rate of 10°C/min. The gas used was nitrogen with a purging rate of 30 ml/min (Plessis et al., 1996)

Stability Study

Stability studies were carried out to investigate the leaching of drug from formulation during storage. The ability of vesicles to retain the drug was assessed by keeping the selected elastic vesicular suspension in sealed glass bottle (25 ml capacity) at 25 ± 2 °C, and 4–8 °C for 3 months. Samples were withdrawn periodically and analyzed for aggregation and residual drug content. The initial entrapment and drug content was considered as 100% (Munish et al., 2008)

Corneal Permeability Studies

Whole eye ball of goat was transported from the local butcher shop to the laboratory in cold (4 °C) normal saline within one hour of slaughtering of the animal. The cornea was carefully excised along with 2 to 4 mm of surrounding scleral tissue and was washed with cold normal saline till the washing was free from protein. Isolated cornea was mounted by sandwiching surrounding scleral tissue between clamped donor and receptor compartments of an all glass modified Franz diffusion cell in such a way that its epithelial surface faced the donor compartment. The corneal area available for diffusion was 0.78 cm^2. The receptor compartment was filled with 11 ml of freshly prepared Phosphate buffer (pH 7.4). 1 ml of test formulation was placed on the cornea. Evaporation of the test formulation was prevented by sealing the opening of the donor compartment with a glass cover slip, while the receptor fluid was maintained at 35 °C with constant stirring, using a Teflon-coated magnetic stir bead. 1 ml sample was withdrawn from the receptor compartment at various time intervals up to 120 min and withdrawn samples were replaced with equal volume of Phosphate buffer. The samples were analysed for Terbinafine hydrochloride by measuring absorbance at 283.4 nm in a UV spectrophotometer. At the end of the experiment, each cornea, freed from sclera, was weighed, soaked in1 ml methanol, dried overnight at 90 °C and reweighed. From the difference in weights corneal hydration was calculated.

used at a concentration of 10 mg/ml for the preparation of vesicles (Ballie, 1985)

Antifungal Activity

Terbinafine hydrochloride is fungistatic and inhibits the biosynthesis of ergosterol, the major sterol found in the fungal cell membrane. The antifungal activity of Terbinafine hydrochloride from the SEVs as well as the reference standard (Terbinafine hydrochloride dissolved in 30% v/v of ethanol) was determined using *Candida albicans* as a representative fungi, adopting the cup plate method. The mean inhibition zone was calculated, and the value was taken as an indicator for the antifungal activity (Shilpa and Indu 2012)

***In vivo* Safety Studies**

Safety assessment for ocular application was approved by the School of Pharmacy & Technology Management, Institutional Animals Ethics Committee - Reg. No. *1300/ac/09/CPCSEA* and the approval No. was SPTM-IAEC/Nov-3/06/20 (Appendix I). Also, performed as per the details below.

1. **Dermal Irritation/Corrosion Test as per OECD Guideline 404**

According to the OECD guideline 405, before studying the in vivo eye irritation/corrosion test, the in vivo dermal effects of the substance should be conducted and evaluated in accordance with the OECD Testing Guideline 404. The albino rabbit is the preferable laboratory animal. The substance to be tested is applied in a single dose to a small area of skin (approximately 6cm²) of an experimental animal; untreated skin areas of the test animal serve as the control. The exposure period is 4 hours. Residual test substance should then be removed. The dose is 0.5ml (liquid) applied to the test site. The method consists of two tests: the initial test and the confirmatory test (used only if a corrosive effect is not observed in the initial test). All animals should be examined for signs of erythema and oedema during 14 days. The dermal irritation scores should be evaluated in conjunction with the nature and severity of lesions, and their reversibility or lack of reversibility. When responses persist to the end of the 14-day observation period, the test substance should be considered an irritant (OECD Test Guideline, 2002)

2. Eye Irritation/Corrosion Test as per OECD Guideline 405

The test substance is applied in a single dose in the conjunctival sac of one eye of each animal. The other eye, which remains untreated, serves as a control. The initial test uses an animal; the dose level depends on the test substance nature. A confirmatory test should be made if a corrosive effect is not observed in the initial test, the irritant or negative response should be confirmed using up to two additional animals. It is recommended that it be conducted in a sequential manner in one animal at a time, rather than exposing the two additional animals simultaneously. The duration of the observation period should be sufficient to evaluate fully the magnitude and reversibility of the effects observed. The eyes should be examined at 1, 24, 48, and 72 hours after test substance application. The ocular irritation scores should be evaluated in conjunction with the nature and severity of lesions, and their reversibility or lack of reversibility. A *chronic repeat dose study* in which five repetitive doses of the vesicular dispersion were instilled every day, in the conjunctical sac at an interval of 5 min, for a period of one week were also performed. In order to evaluate whether the vesicular system was safe for long term therapy or not. Animals were evaluated for ocular irritation/corrosion based on scale as per OECD guidelines (405) (OECD Test Guideline, 2002).

Table 1 - Result of Drug Excipients Compatibility Study after 1 Month

Drug + Excipient	Ratio	At Room Temperature	In Freezer	40°C±2°C, 75%±5% RH
TH	1	3 Weeks	3 Weeks	3 Weeks
TH + Span 20	1:1	3 Weeks	3 Weeks	3 Weeks
TH + Tween 80	1:1	3 Weeks	3 Weeks	3 Weeks
TH + Span 20 + Tween 80	1:1:1	3 Weeks	3 Weeks	3 Weeks

(TH: Terbinafine Hydrochloride, Here drug and excipients kept with different ratio at three conditions for 3 weeks)

Table 2 - Mixture Design Layout

Run	Component A Span 20 (%)	Component B Tween 80 (%)	Response 1 Particle Size (µm)	Response 2 Drug Content (%)
1	30.000	70.000	6.5	78.5
2	50.000	50.000	3.3	82.8
3	40.000	60.000	7.9	73.7
4	50.000	50.000	2.4	83.2
5	30.000	70.000	7.4	79.5
6	60.000	40.000	6.9	75.2
7	70.000	30.000	8.2	73.5
8	70.000	30.000	6.7	72.4

(The using of Design expert 8 with 2 factorial design that independent factors were Span 20 and Tween 80 and the dependant factors were particle size (µm) and drug content (%) gave optimized results on bases of given trails results. From that result of 4 indicated best optimized result.)

RESULTS

Preformulation Studies of Pure Drug

Identification of pure drug: It was carried out by FTIR spectroscopy. (Figure 1 and Figure 2)

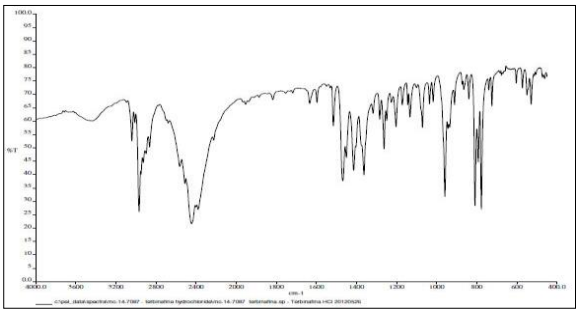


Figure 1 - FTIR Spectra of Standard Drug
(Infrared spectra of standard drug gave different functional group IR range with wavenumber which was present.)

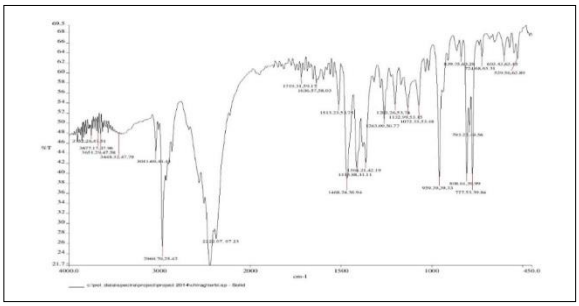


Figure 2 - FTIR Spectra of Sample Drug
(Spectrum of sample drug indicated with different peaks on graph that observed value compare with the standard and identify the drug.)

Table 3 - IR Spectra having Wave Number Range which shows Signal Assignment

Sr. No.	Functional Group	Wavenumber (Cm^{-1})	Observation (Cm^{-1})
1.	OH stretching	3200-2800	2969.70
2.	CN stretching	2400-2000	2222.07
3.	C=O streching	1800-1600	1636.57
4.	CH bending	1600-1400	1468.24
5.	COOH streching	1400-1200	1364.21
6.	CH bending	1000-800	808.61
7.	C-Cl streching	800-600	777.53

(The table depicts the functional group and their wavenumber range of standard drug which was compared with the observed value of sample drug.)

The drug sample was firstly analyzed by FTIR (Fourier transform infrared) with standard sample. The result indicated that Terbinafine hydrochloride was pure and free from impurities because the value of drug sample similar with standard value. (Table 3)

Melting point determination: Melting point of Terbinafine hydrochloride was found to be in the range of 195°C to 198°C with decomposition as reported in Pharmacopoeia, thus indicating purity of the drug sample. (Table 4)

Absorbance: The maximum absorbance of drug was determined by UV spectrophotometer and was found the maximum wavelength at 283. 4 nm that is similar with the standard absorbance. (Figure 3)

Table 4 - Melting Point Determination of Drug

No.	Melting point (°C)	Average Melting point (°C)
1.	194°C to 199°C	195°C to 198°C
2.	193°C to 197°C	
3.	197°C to 200°C	

(Melting point of Terbinafine hydrochloride reported three times and made average of them which was considered on bases of Pharmacopoeial value.)

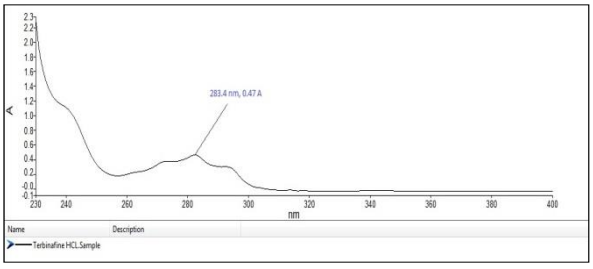


Figure 3 - UV Graph of Pure Drug (The absorbance of sample drug determine by the UV spectrophotometer that was found to be 283.4 nm.)

Callibration Curve of Terbinafine Hydrochloride:

Appropriate volume of aliquots from standard Terbinafine Hydrochloride stock solutions were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 2 to 20µg/ml. Absorbance at 282 nm was measured and the plot of absorbance vs. concentration was plotted.

The straight-line equation was determined. From the optical characteristics of the method, it was found that Terbinafine Hydrochloride obeys linearity within the concentration ranges 2-20µg/ml The result indicated that Terbinafine hydrochloride complies the Lambert - Beer's law between the concentration ranges of 2-20 µg/ml. The R^2 value was found to be 0.999. (Figure 4). **Solubility Study:** Qualitative solubility of drug was checked in various solvents and found that the drug was freely soluble in methanol and insoluble in chloroform, sparingly soluble in 0.1N HCl, slightly soluble in water, the result was found that the drug Terbinaine Hydrochloride is lipophillic in nature because it was soluble in organic solvent (Table 6)

Drug - Excipient Compatibility Studies

The results of compatibility study are shown in Table 7.

Design Expert Output

The observed graphs from Design Expert version 8.7.1 shown below:

For an initial characterization of the vesicles, Terbinafine hydrochloride loaded SEVs were examined microscopically (Motic microscope, magnification 100 X). Optical inspection indicated the vesicles to be small in size, round in shape, bilamellar/multilamellar (Figure 5) and no aggregation irregularities were observed in the system.

Table 5 - Callibration Curve of Terbinafine Hydrochloride in Methanol at λ_{Max} 283 Nm

Sr. No.	Concentration (µg/ml)	Absorbance
1.	2	0.054
2.	4	0.119
3.	8	0.286
4.	16	0.628
5.	20	0.840

(Calibration curve measured for the comparison with of sample drug concentration and these samples was prepared in methanol at λ_{Max} 283 Nm.)

Table 6 - Solubility Study of Terbinafine Hydrochloride in Different Solvents

Sr. No	Solvent	Inference	Observed Solubility
1.	Methanol	++++	Freely Soluble
2.	Ethanol	+++	Soluble
3.	Water	+	Sparingly Soluble
4.	0.1 N HCL	+	Sparingly Soluble
5.	Chloroform	---	Insoluble

(From the table solubility study of Terbinafine hydrochloride obtained with the different solvent involved for the solubilisation of drug.)

Table 7 - Result of Drug - Excipients Compatibility Study after 1 Month

Condition	Time duration	Ratio of Ingredients (1:1)			
		TH	TH + Span 20	TH + Tween 80	TH + Span 20 + Tween 80
40 ± 2°C/75 ± 5% RH	1 week	No change	No change	No change	No change
	2 week				
	3 week				
At room temperature	1 week	No change	No change	No change	No change
	2 week				
	3 week				
In freezer	1 week	No change	No change	No change	No change
	2 week				
	3 week				

(TH: Terbinafine Hydrochloride,The compatibility study at the given ratio of ingredients provided the result on the bases of IR spectra taken at every weekly form. There was no change found at the range of wavenumber which actually present the all functional groups.)

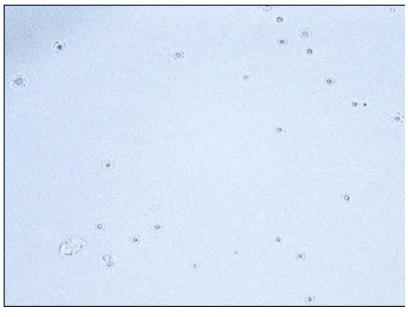
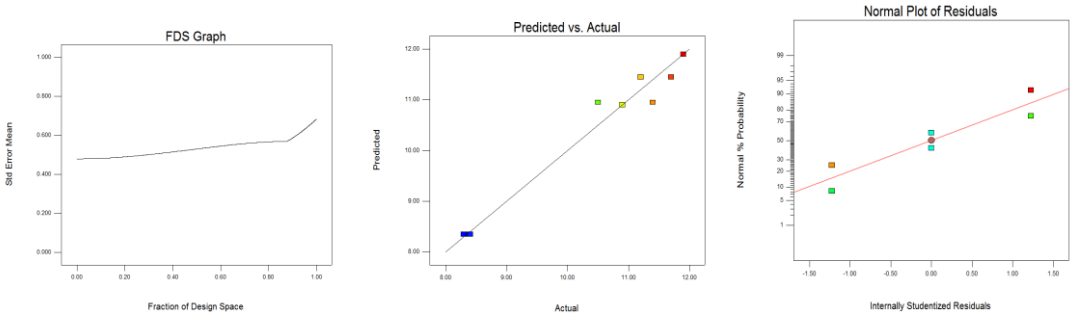
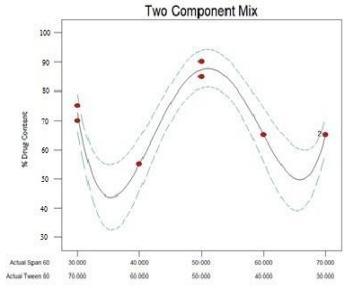


Fig 5: Morphology and Vesicle Size
(The motic image of terbinafine hydrochloride loaded vesicles with 100 X magnification of indicated vesicles size and shape which was uniform.)



(a) **(b)** **(c)**



(d)

Figure 6 represent the plots (a) FDS graph (b) Predicted vs. Actual (c) Normal plot of residuals (d) Two component mix. Shown here (a) Fraction of design space indicated to the standard deviation of error to the dependant factors which fluctuated least. (b) The prediction was based on the trails results shown as dark red dots nearer to straight line that was added into the given trails table. From design expert adjusted with actual for optimization. (c) The normal probability plot is a graphical tool for comparing a data set with the normal distribution. These dots indicates probability of similar optimization. The standardized residual of the linear regression model and see if the error term is actually normally distributed and such way that it should be straight line.(d) These was final two component mix of independent factors vs. total drug content that was best as the optimized result at higher peak.)

Table 8 - Calibration Curve of Terbinafine Hydrochloride in Phosphate Buffer (pH 7.4) at λ_{Max} 283.4 Nm

Sr. No.	Concentration (µg/ml)	Absorbance
1.	2	0.024
2.	4	0.039
3.	6	0.055
4.	8	0.073
5.	10	0.089

(The calibration curve of terbinafine hydrochloride in phosphate buffer pH 7.4)

The average size of the vesicles under the Malvern zeta sizer was about 328.4 nm (Figure 7)

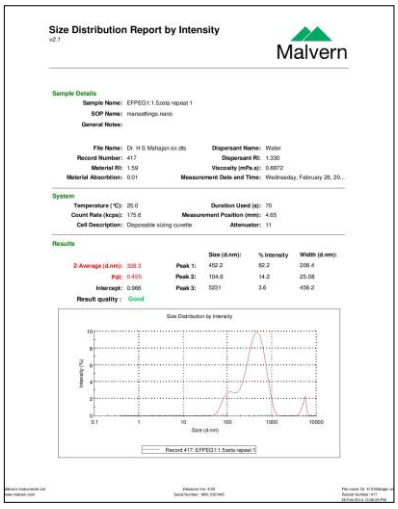


Fig 7 - Size distribution by Malvern Zeta Size Analyzer
(The vesicles particle size analysed by Malvern zeta sizer devoted three peaks, in which maximum peak intensity above 80% had average size 328.3 nm that shown good quality result.)

Figure 8 - Result of Malvern Zeta Potential Analyzer
(The result of zeta potential was -17 that indicated system was thermodynamically stable with low scope of coalescence and maximum peak area 100% at that potential by Malvern zeta analyser.)

Zeta Potential

Zeta potential of the optimized formulation was measuredusing Malvern's zetasizer at 25°C. It was found tobe -17. The negative value indicates a lowscope of coalescence of vesicular system and confirmed as in nano range (-30 to 30). (Figure 8)

Total Drug Content

Actual amount of Terbinafine Hydrochloride added for all practical purposes into the vesicular dispersions was 10.0 mg/ml and the drug content of the optimized formulation was found to be 8.32 mg/ml that means 83.2% based on the calibration curve.

Differential Scanning Calorimetry (DSC)

The occurrence of any interaction between a drug and excipients in the formulation can be predicted by conducting the differential scanning calorimetry studies. The thermograms of final formulation display the characteristic features of the drug. This indicates no possible interaction between the excipients and Terbinafine Hydrochloride. The results are shown in figure no: 9. DSC is a fast andreliable method to screen drug-excipient interactions as indicated by appearance of a new peak(s), change in the peak shape and its onset, peak temperature/melting point and relative peak area or enthalpy. Terbinafine hydrochloride with all the excipients showed a sharp endothermic peak at 200.2 ^{0}C. Pure Terbinafine hydrochloride had sharp endothermic peak at 196.8 ^{0}C (m.p. 195-198 ^{0}C). Drug loaded SEVs showed a broad endothermic good interaction of all components due to increase in phase transition temperature of SEVs upon loading of drug.

Stability Study

The results of stability studies are compiled in Table 8. Stability of vesicles are referred to in terms of aggregation/irregularity of vesicles over a period of 3 months of storage. Extent of drug aggregation upon storage in refrigerator and at ambient room temperature was significantly low. Hence the formulation can be refrigerated or stored at room temperature for use.

Table 9 - Result of Stability Study

Condition	Time	Appearance	Aggregation
Refrigeration (4-8 ^{0}C)	Day 0	Clear solution	No
	3 Month	No change	No
Ambient Stability Temperature (40 ^{0}C ± 2 ^{0}C, 75% RH ± 5%)	Day o	Clear solution	No
	3 Month	No change	No

(The result of stability study of vesicle system for two conditions and analyzed the appearance and aggregation by visual inspection after one month.)

Corneal Permeability Studies

The result of the corneal permeability study is comprised in the table no. 5.8. The maximum drug release observed at 150 minutes is about 82.50% (Table 10). The results also specified that the concentration of Terbinafine Hydrochloride in cornea remained fairly constant for up to 2.5 hr.

Table 10 - Results of Corneal Permeability Study

Sr. No.	Time Point (min.)	Absorbance	% Drug Permeated
1	30	0.012	11.23
2	60	0.028	31.29
3	90	0.040	46.25
4	120	0.056	66.26
5	150	0.069	82.50

(There was drug permeated increases with the time and gave 80% above at 150 min.)

After end of experiment, Initial weight of cornea was to be found 0.920 gm. and after overnight dried it was found to be 0.180 gm. The difference was 0.74 gm that indicated 80.43% corneal hydration.

Figure 9 - Corneal Permeability Studies of Goat Eye (a) Dissection of eye (b) Diffusion study on magnetic stirrer

Antifungal Activity

The mean zone of inhibition, of sample SEVs for (C) that was 2.80 cm and for (A) 2.60 cm. It is more or less same as that of the Reference standard of Terbinafine Hydrochloride (B) that 3.10 cm. It is to be noted that sample used in the study was confirmed to show antifungal activity. There is no significant difference in zone of inhibition obtained from the formulation SEVs in comparison to the reference standard (alcoholic solution). Hence achieving equally good zones of inhibition as the alcoholic solution of Terbinafine Hydrochloride with the vesicular dispersion was a significant achievement.

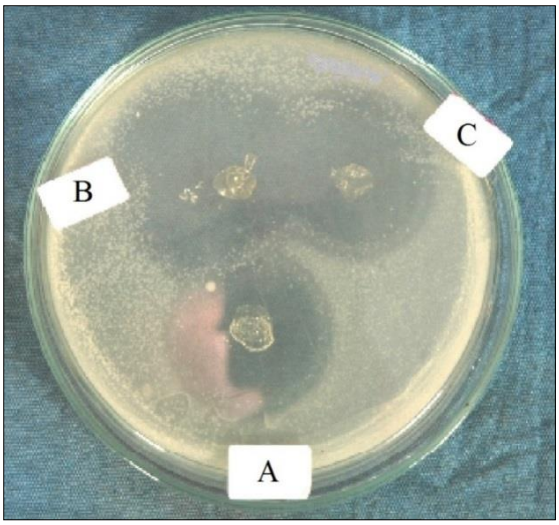


Figure 10 - Antifungal Activity of Formulation and Reference Standard
(The image indicated three zone of inhibition that A and C for same sample and B for reference standard for the comparison of antifungal activity.)

In vivo safety studies

Dermal Irritation/Corrosion Test

Non-irritant/corrosive nature of developed SEVs when applied to dermal tissues was confirmed as indicated by 0/3 score for erythema and oedema each. Hence they may be considered safe for dermal application.

Chronic repeat instillation irritation/corrosion test: The treatment of ocular fungal infection involves long term therapy, so we evaluated the developed formulation(s) for chronic repeat instillation. Again a zero score was observed for eye irritation/corrosion study which clearly depicted that SEVs were non-irritant even upon chronic use to all ocular tissues. In view of the zero score obtained in the study the developed SEVs formulation may be considered safe for chronic ocular use.

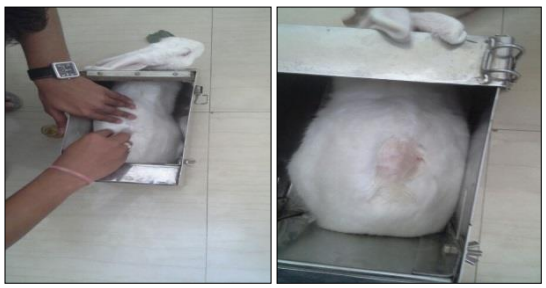


Figure 11 - Dermal Irritation Test on Rabbit skin
(The image for the irritation test provided by the 3 cm^2area and rest of was untreated skin for the comparison and rabbit put into the cage.)

Figure 12 - Eye Irritation Test on Rabbit (Control and Standard Sample Eye)
(The image for the eye irritation test with the comparison for control and standard eye as right to left eye)

Acute Eye Irritation/Corrosion Test

A score of 0/3 for single and 0/3 for repeat instillation of optimized formulation(s) establishes that developed SVs are completely safe for ocular use (Table 11).

Table 11 - Result of Eye Irritation Test

Sr. No.	Time Point (Hr.)	Inflammation	Redness
1	1	0	0
2	24	0	0
3	48	0	0
4	72	0	0

(Score: 0-None, 1-Mild, 2-Moderate, 3-Sevear, The table for inflammation and redness measurement to the 1, 24, 48, 72 hours with regarded score.)

DISCUSSION

Drug delivery into the posterior segment of the eye is complicated by the existence of the blood-ocular barrier. Available strategies for delivering drug to posterior segment via topical route are generally invasive and costlier. Because of this purpose, we make an effort to develop non-ionic surfactant based elastic vesicles by using Span 20 and Tween 80. It was also evaluated for targeting posterior eye segment.

The developed ophthalmic formulation particle size should be less than 10 μm for avoidance of eye irritation. The nano-sized particles represent a state of matter characterized by more prominent bio-adhesion and more surface area available for association between the cornea and conjunctiva in ophthalmic drug delivery systems.

The lipid vesicles with higher ethanol concentration, reduces the membrane thickness of vesicles due to the formation of a phase with penetrating hydrocarbon chains. Also, ethanol may stabilize steric charge that lead to decrease in the particle size. A nano size vesicle helps to across the anatomical constraints in the eye.

A nano size vesicle has a contributed advantage for treatment of superficial fungal infections. Even though the pore size of bio-membranes is expected to be much smaller than the vesicle size simply the surfactant nature of the vesicles may temporarily increase the pore size such that slightly bigger vesicles may also crush in.

The elasticity of developed formulation may be imputed to the presence of tween 80 as an edge activator (EA). EAs are often a single chain surfactant, which not stabilizes the vesicles and increases the deformability of the bilayer by lowering the interfacial tension. Probably the presence of tween 80 reduced the transition, which reflects the presence of a system having different degrees of disruption in packing characteristics.

Corneal permeation data suggests that developed formulation is improved in targeting the posterior segment of eye. The SEVs had better retention and more persistent interaction with the ocular surface. If the drug is crossing the intact tissue it will surely cross the inflammatory tissues with a greater concentration. The prolonged ocular retention of the SEVs is in good agreement that showed prolonged corneal retention of colloidal particles. Penetration of drug was more incase of nanoparticle may be due to higher permeation and zeta potential of this nanoparticles.

There was an equally good zone of inhibition of the alcoholic solution of Terbinafine Hydrochloride with the developed vesicular system that was a significant achievement. This study was showed to confirm the antifungal activity of developed formulation.

The developed formulation was found to be safe for ocular treatment due to the study of in vivo safety studies. It is used to treat internal eye fungal infections like endophthalmitis, chorioretinitis and Chlamydial infections for which presently only invasive intravitreal injections of the active therapeutic are available.

CONCLUSION

The formulation optimized by the 2 factorial designs of Design Expert 8 and response surface plots obtained for each dependant factors.

From the obtained results, it shows suitability of formulation in terms of stability and therapeutic efficacy. Hence it could be a breakthrough for effective delivery of drugs into posterior eye.
The nanorange of formulation could be potential carriers for targeting the posterior segment of the eye. The nanoparticles may represent an interesting vehicle in order to enhance the therapeutic index of clinically challenging drugs with potential application at ocular level.

The corneal permeation data suggests that developed formulation is improved in targeting the posterior segment of eye. The developed formulation showed better retention and more persistent interaction with the ocular surface.

Results of anti-fungal studies showed that there was no significance difference in zone of inhabitation obtained by developed formulation in comparison to the reference standard that confirmed the antifungal activity of developed formulation.

The formulation was found to be non-irritating with no ocular damage. It may thus be concluded that the developed systems showed great assure, with their novel being approach. The established safety added to the confidence for use in ocular and topical routes. The low cost of ingredients of these vesicular systems is also of keen significance. Moreover, the method of preparation is extremely self-generated, and hence it will be easy to scale-up and commercialize these vesicles.

In conclusion, we suggested that surfactants based elastic vesicular drug carrier system could be promising vehicles of hydrophobic drug delivery to the ocular posterior segment.

REFERENCES

ADI, Acute dermal irritation/corrosion. In: *OECD (Ed.) 2002*; Test Guideline, vol. 404.

AEI, Acute eye irritation/corrosion. In: *OECD (Ed.) 2002*; Test Guideline, vol. 405.

Akash D, Bhanu T. (2013) Development and Evaluation of Terbinafine hydrochloride for Tablet Formulation.*International Journal of Pharmaceutical and Biological Science 1: 36-52.*

Ballie AJ. (1985) The preparation and properties of niosomes- non-ionic surfactant vesicles. *J. Pharm. Pharmacol 37: 863-68.*

Davis JL, Gilger BC, Robinson M.(2004) Novel approaches to ocular drug delivery. *Current Opinion Molecular Therapeutics 6: 195–05.*

Hillgren A, Evertsson H, Aldén M. (2002) Interaction between lactate dehydrogenase and tween 80 in aqueous solution. *Pharm. Res. 19: 504–10.*

Indian Pharmacopoeia, (2010) Controller of Publication, Govt. of India, Ministry of Health and Family Welfare, New Delhi. 1: p 301.

Kaur IP, Garg A, Singla AK, Aggarwal D. (2004) Vesicular systems in ocular drug delivery: An overview. *International Journal Pharmaceutics. 269: 1-14.*

Lang JC, (1995) Ocular drug delivery conventional ocular formulation. *Advanced Drug Delivery Review 16: 39-43.*

Munish A, Gurmeet S, Dipak K. (2008) Effect of Formulation Parameters on Corneal Permeability of Ofloxacin, *Sci Pharm. 76: 505–14.*

Plessis J, Ramachandran C, Weiner N, Moiler, DG, (1996) The influence of lipid composition and lamellarity of liposomes on the physical stability of liposomes upon storage. *Int. J. Pharm. 127: 273–78.*

United States Pharmacopoeia/National Formulary, (2000) 24th ed. Rockville, MD: Pharmacopeial Convention, 2149.

Sahoo SK, Dilnawaz F, Krishnakumaran S. (2008) Nanotechnlogy in ocular drug delivery. *Drug Discovery Today.13: 144-51.*

Shilpa K, Indu P K. (2012) A novel nanovesicular carrier system to deliver drug topically. *Pharmaceutical Development and Technology 1–13.*

Zimmer A, Kreuter J.(1995) Microspheres and nanoparticles used in ocular delivery systems. *Advanced Drug Delivery Reviews. 61-73.*