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The Effect of Surfactant on Developed Ophthalmic Drug Delivery System for the Delivery of Antifungal Drug in Posterior Segment of Eye

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Abstract*—Eye is a complex organ; drug delivery to the posterior segment poses significant challenges due to its unique anatomical and physiological barriers. The surfactant based drug delivery system enhances the bioavailability by increasing drug solubility, prolonging pre-corneal retention and enhancing permeability. So by considering the advantages of surfactant based drug delivery system, this study were carried out on Voriconazole; a lipophilic category antifungal drug. The work describes usefulness of a surfactants based drug delivery system for targeting topically applied drug to the posterior segment of the eye. The system was formulated using Span 80 with (edge activator) Tween 80 by using ethanol injection method. A 32 full factorial design was used to study the effect of two independent variables, namely, the concentration of Span 80 and the concentration of Tween 80. For optimization of formulation drug content and excised goat eye permeability were taken as response variables. Formulation batch containing 20:80 (Span 80: Tween 80) shown better corneal permeability performed on goat's eye and higher drug content amongst all formulated batches. Therefore, the study confirmed that surfactant based ocular drug delivery systems shows good permeability for ocular delivery of Voriconazole and it can be used to deliver drugs to the posterior segment of the eye.*

Keywords*—Permeability; surfactant based ocular drug delivery; Voriconazole.*

I. INTRODUCTION

Ocluar drug delivery is one of the most challenging tasks for the Pharmaceutical researchers. To maintain a therapeutic level of the drug at the site of action for a prolonged duration is major obstacle. Due to unique anatomy and physiology, the eye presents challenges for delivery of pharmaceuticals [1]. There are varieties of physiological barriers affected to the eye. It requires site-specific drug delivery systems to target the vitreous cavity, retinal pigment epithelium and choroid. Diseases affecting the posterior segment are age-related macular degeneration, cytomegalovirus, uveitis and fungal infections which can cause irreparable vision loss due to inadequate drug levels arising from poor delivery [2].

Endophthalmitis means fungal infection inside the eye involving the vitreous and/or aqueous humors. Mostly amphotericin B and triazoles are used for its treatment. The intraocular penetration of amphotericin B after topical or systemic treatment is limited and produces retinal toxicity. In addition, many fungal pathogens affecting the human eye are not susceptible to these agents. Recently developed second-generation triazolederivates (eg, voriconazole) seem to be promising alternatives. Voriconazole can be given either systemically or intravitreally. It penetrates well into the ocular tissue after systemic administration and shows less systemic side effects compare to amphotericin B [3].

Here topical drug delivery is most preferred due to its patient compliance. However, topical application is associated with many other complications, such as extensive precorneal drug loss by high tear fluid turnover, non-productive absorption, drainage through the nasolacrimal duct, impermeability of the corneal epithelium, transient precorneal residence time and metabolism of the drug by anterior segment enzymes [2]. It prevent drug from achieving therapeutic concentration levels at target tissues so recently the drug delivery through vesicles has gained lot of attention due to its prominent advantage in improving bioavailability and reducing dose frequency. The use of non-ionic surfactant enhances the bioavailability by increasing drug solubility, prolonging pre-corneal retention and enhancing permeability [4].

The objective of study was to formulate surfactant based ocular drug delivery system using Voriconazole as drug candidate, to study the effect of different concentrations of span and tween in formulations and to evaluate surfactant based formulation by various parameters to increase the corneal permeability of drug by using a surfactant based novel vesicular system.

II. MATERIALS AND METHODS

Material

Tween 80, Span 80 and ethanol were procured from S.D lab chemical center, Mumbai. Voriconazole was a kind gift sample from Cipla lab, Mumbai.

Methods

Preformulation studies

Characterization of drug.

A. Melting point

Melting point of Voriconazole was determined by micro-controlled based melting point apparatus (Chemiline-CL 726).Taking a pinch of Voriconazole into a capillary tube, closed at one end. Then the capillary was inserted in bath of silicone oil which was heated in controlled manner with the help of electrical heating coil. The temperature which made drug sample transparent was noted as melting point temperature. Average of triplicate readings was noted and compared with the literature value.

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B. Differential Scanning Calorimetry (DSC):

DSC was performed in order to assess the thermal behavior of the drug. It measures heat flow in and out of both sample and reference during control temperature. About 1 mg of the sample was sealed in the aluminium pan and heated at the rate of 10°C/min, covering a temperature range of 30°C to 350°C under nitrogen atmosphere, at flow rate of 20 ml/min [5].

C. FTIR Spectroscopy

IR study was carried out to check purity of drug. It was determined by Fourier Transform Infrared spectrophotometer. The IR spectrum of Voriconazole was recorded using Fourier transform infrared spectroscopy (FTIR) to check its purity. The spectrum was recorded over the wave number of 4000 to 400 cm-1[6].

D. λ max Determination

Accurately weighed 100 mg of drug was dissolved in 100 ml of ethanol to form stock-I (1000µg/ml). 10 ml of solution was withdrawn from stock-I added to 100 ml of volumetric flask. The volume was made to 100 ml using ethanol to form stock-II (100 µg /ml). Then 1 ml from stock-II was withdrawn and added to 10 ml volumetric flask. The volume was adjusted to 10ml using ethanol to prepare final solutions (10µg/ml). The UV spectrum was recorded in range of 200-400 nm using UV spectrophotometer. The λ max was determined by scanning solution of 10µg/ml against blank solution.

E. Drug Excipient Interaction Study

Drug- excipient interaction study was performed by FTIR and DSC studies. IR was determined by Fourier Transform Infrared Spectrophotometer (FTIR-410, Jasco, Japan). The spectra were scanned over wavelength region of 4000 to 400 cm-1 at resolution of 4 cm-1. For DSC study, about 1mg of the sample was sealed in the aluminum pan and heated at the rate of 10˚C/min, covering a temperature range of 30˚C to 200˚C under nitrogen atmosphere, at flow rate of 20 ml/min [5].

Characterization of excipients

F. Saponification Value

2 gm. of sample accurately weighed and transferred into 200ml borosilicate glass flask fitted with reflux condenser. Then in this flask 25ml of 0.5M ethanolic KOH and little pumice powder was added. All this material was boiled under reflux on water bath for 30min. 1ml of phenolphthalein solution was added and this whole solution was titrated with 0.5M HCl (a). Blank titration was carried out (b). Saponification value was determined by using formula-

$$\text{Saponification Value} = 28.05 \times (b-a)/W$$

Where, W- Weight in gm of substance [8]

G. Acid Value

Acid value is the number which express in milligrams. 10 g of the substance under examination was accurately weighed into 50ml flask of a mixture of equal volume of ethanol (95 %) and ether previously neutralized with 0.1 m potassium hydroxide to phenolphthalein solution. Phenolphthalein solution was added (1ml) and titrated with 0.1 KOH until the solution remains faintly pink after shaking for 30 seconds. Acid value was determined by using formula-

$$\text{Acid value} = 5.61 \times n/W$$

Where, n= the number of ml of 0.1 M KOH required.

W= the weight in g of the substance [8].

III. Preparation of Surfactant Based Vesicles

Surfactant based vesicles containing Span 80 and edge activator (EA) Tween 80 were prepared by ethanol injection method. Voriconazole and span 80 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 1000 rpm/ min. Voriconazole was used at a concentration of 10 mg/ml for the preparation of vesicles [9].

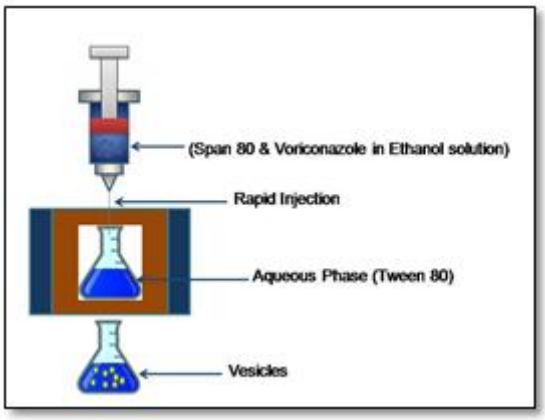

Fig. 1. Ethanol injection method for formulation of surfactants based ocular drug delivery system of voriconazole.

A. Design of Experiment

A 3^2full factorial design was chosen in which concentration of tween 80 (X1) and concentration spans 80 (X2) were used as 2 factors and experimental trials were performed at all 9 possible combinations.

TABLE I. Amount of variables in 3^2 factorial design batches.

Coded Values	Actual Values	
	X1: (Conc of Span 80)	X2:(Conc of Tween 80)
-1	10 %	70 %
0	20 %	80 %
1	30 %	90 %

Responses:
X1= Total drug content
X2= Corneal Permeability Studies (CADD/ cm^2)

TABLE II. A 3^2 factorial design layout.

Formulation batches	Coded values	
	X1	X2
F1	1	1
F2	1	-1
F3	1	0
F4	0	0
F5	0	-1
F6	0	1
F7	-1	0
F8	-1	1
F9	-1	-1

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TABLE III. Composition of surfactant based vesicles of Voriconazole.

Formulation Batch	Ingredients				
	Drug (mg)	Span 80 (%)	Tween 80 (%)	Ethanol (ml)	Water (ml)
F1	100	30	90	q. s	q. s
F2	100	30	70	q. s	q. s
F3	100	30	80	q. s	q. s
F4	100	20	80	q. s	q. s
F5	100	20	70	q. s	q. s
F6	100	20	90	q. s	q. s
F7	100	10	80	q. s	q. s
F8	100	10	90	q. s	q. s
F9	100	10	70	q. s	q. s

IV. EVALUATION OF FORMULATION

A. *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 255 nm [2].

% Drug content = actual drug content/Theoretical drug content*100

B. *Corneal Permeability Studies*

Study is performed on a Franz diffusion cell assembly. Drug permeation studies were carried out by using freshly excised goat cornea. Goat whole eyeballs were transported from the local butcher shop to the laboratory in cold (4°C) normal saline within 1 hour of slaughtering of the animal. The cornea was carefully excised along with 2 to 4 mm of surrounding sclera tissue and was washed with cold normal saline till the washing was free from proteins. The receptor compartment of an all-glass modified Franz diffusion cell was filled with 40 mL freshly prepared normal saline solution (pH 7.4) and all air bubbles were expelled from the compartment. Excised cornea tissue was fixed between clamped donor and receptor compartments in such a way that its epithelial surface countenanced the donor compartment. The corneal area available for diffusion was 2.26 cm2. An aliquot (1 mL) of test solution was placed on the cornea and the opening of the donor cell was sealed with a glass cover slip. The receptor fluid was kept at 37°C with constant stirring using a Teflon-coated magnetic stir bead. Permeation study was performed for 180 minutes and samples were withdrawn from receptor compartment and analyzed for voriconazole content by measuring absorbance at 255 nm in a spectrophotometer. [10].

C. *Morphology*

Vesicles were characterized by using optical microscope for structural attributes such as uniformity of size, shape and physical stability characteristics i.e. aggregation and/or irregularity [2].

D. *Vesicular Size Determination and Zeta potential Measurement*

Particle size analysis was carried out by using Horiba Nanoparticle Analyzer SZ- 100 instrument. SZ-100 uses the technique of dynamic light scattering to determine particle size. During testing, temperature was maintained at 250 C. Sample was placed in sample holder. Zeta potential of formulation can be measured by zeta meter. Zeta potential analysis was determined by using Horiba Analyzer SZ-100 instrument [9].

E. *Stability Study*

Stability studies were carried out for optimized formulation. For stability studies the optimized formulations were stored at temperature 4˚C-8˚C and 25˚C for a period of 2 months. Sample was analyzed for the change in appearance and aggregation [2]

V. RESULTS

Preformulation Studies

Characterization of drug.

A. *Melting point:* Melting point of Voriconazole was found to be in range of 127-130°C which complies with that given in the literature.

B. *Differential Scanning Calorimetry:* According to the thermogram, a sharp endothermic peak was observed at 130.99 ºC which corresponds to the melting point of pure drug.Such an endothermic peak was also reported for standard drug material near to the melting range. This indicated that Voriconazole drug was in pure form [5].

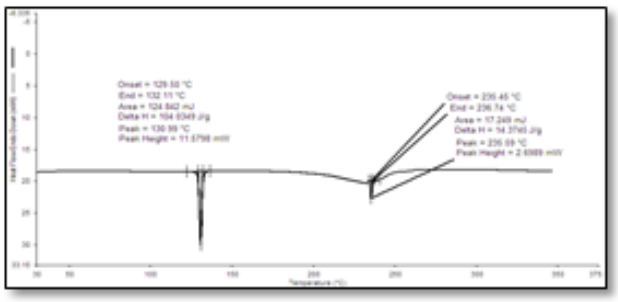

Fig. 2. DSC thermogram of Voriconazole drug.

C. *FTIR Spectroscopy:* The infrared spectrum of Voriconazole was recorded and spectral analysis was carried out which is shown in Fig-3

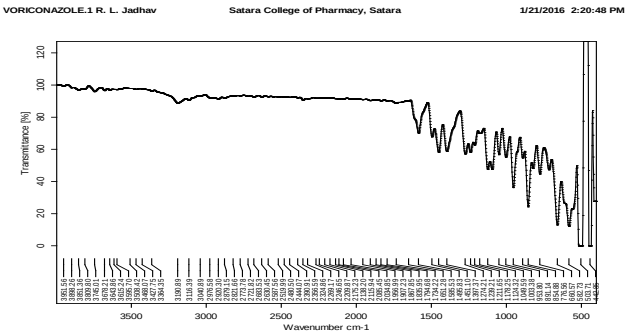

Fig. 3.FTIR spectrum of Voriconazole drug.

D. *λ max determination:* The λ max value of Voriconazole was found to be 255 nm in ethanol.

E. *Calibration curve of Voriconazole:* The standard calibration curve for Voriconazole in ethanol was plotted

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by using following results of absorbance at various concentrations.

TABLE IV. Observations for calibration curve of Voriconazole.

Sr.no	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.2898
3	12	0.3543
4	14	0.4321
5	16	0.4982
6	18	0.5687
7	20	0.6254
8	22	0.6532
9	24	0.7132

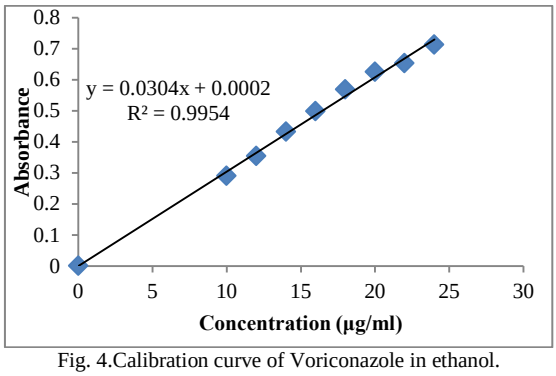

Fig. 4.Calibration curve of Voriconazole in ethanol.

F. Drug excipient interaction study:

This study was carried out to check for any possible interaction between the drug and excipient. Drug – excipient interaction study was performed by FTIR and DSC study.

FTIR Study

From FTIR study, it was found that the peaks found in pure drug and formulation was similar. Thus incorporation of drug in surfactants did not change the position of its functional groups. This indicated that there was no interaction between drug and excipients

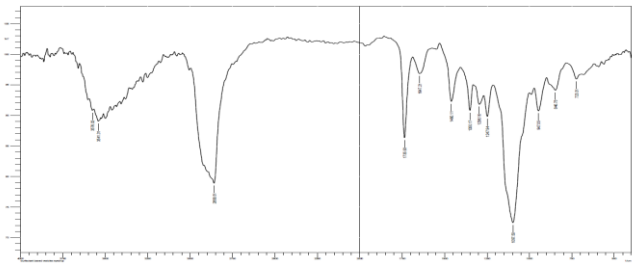
Fig. 5. FTIR spectra of surfactants based ocular drug delivery system of Voriconazole.

DSC Study

According to thermogram, peak was observed at 119.66°C which is at low temperature compared to thermogram of pure drug (130.99 °C). This shifting of peak may be due to presence of surfactants which encapsulates the drug by forming vesicles.

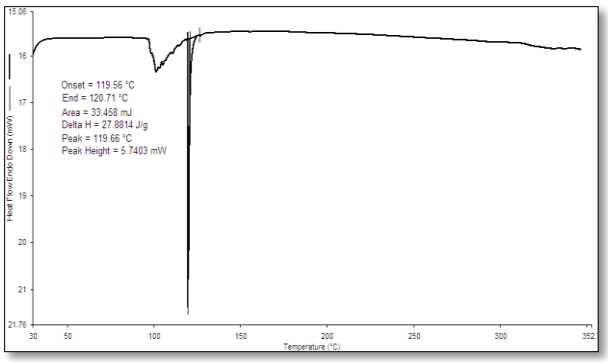

Fig. 6. DSC thermogram of optimized formulation batch.

Characterization of excipient

G. Saponification value

Saponification value was determined by using formula-

$$\text{Saponification value} = 28.05 \times \frac{(b-a)}{w}$$

W- Weight in gm. of substance

- Saponification value for Span 80
  $=28.05\frac{(20.5-10.5)}{2}$
  $= 140.25$

Saponification value of span 80 was found near to standard value (145-160) and it confirmed the purity of span 80.

- Saponification value for Tween 80 = 28.05
  $\frac{(20.5-16.2)}{2}$
  $= 60.30$

Saponification value of tween 80 was found near to standard value (45-55) and it confirmed the purity of tween 80.

H. Acid value

Acid value was determined by using formula-

$$\text{Acid value} = 5.61\frac{n}{w}$$

Where, n= the number of ml of 0.1 M KOH required.
W= the weight in g of the substance.

- Acid value for Span 80 $= 5.61\frac{n}{w}$
  $= 5.61\frac{15.6}{10}$
  $= 8.75$

Acid value of span 80 was found near to standard value (≤ 8) and it confirmed the purity of span 80.

- Acid value for Tween 80 $=5.61\frac{n}{w}$
  $= 5.61\frac{2.5}{10}$
  $= 1.40$

Acid value of tween 80 was found to be complied with standard value (≤ 2) and it confirmed the purity of tween 80.

VI. Evaluation of Surfactant Based Formulation

A. Drug content

Drug content of all the batches of formulation was calculated. Drug content was calculated by using formula

% Drug content = actual drug content/Theoretical drug content*100

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The result was found in the range of 73.72-80.03%. The drug content of the F4 formulation was found to be higher i.e., 80.03% based on the calibration curve. All the values of formulation are shown in table V.

TABLE V. Drug content of all batches of formulation.

Sr. No	Formulation Batch	Drug content (%) ± SD (n= 3)
1	F1	76.26±0.0124
2	F2	76.41±0.0152
3	F3	74.11±0.0100
4	F4	80.03±0.0152
5	F5	77.42±0.0100
6	F6	77.68±0.0152
7	F7	76.21±0.0200
8	F8	78.10±0.0152
9	F9	73.72±0.0200

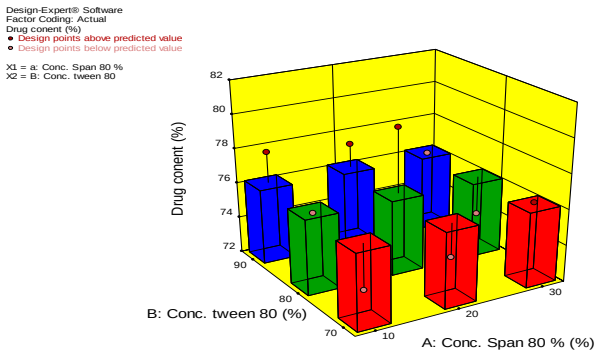

Fig. 7. Response 3D Surface plot for Drug Content (%).

The relationship between the response and variables can be directly visualized from the response surface plot. The response surface plot was generated using Design Expert 10 software and was presented in figures 9. This was used to observe the effect of independent variables on the % drug content. From plot it was observed that batch F4 containing 20:80 (Span 80: Tween 80) shows higher drug content (80.03%).

B. *Corneal Permeability Studies:*

Goat corneas were used to study the permeation across the corneal membrane. In the study, cumulative amount of drug diffused per unit area (CADD/ cm2) for all formulation was calculated.

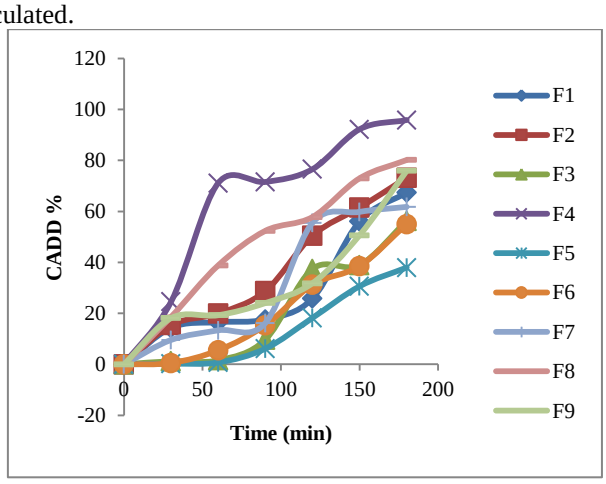

Fig. 8. Comparative drug diffusion profile of F1- F9.

From graphical presentation it was found that batch F4 gives maximum cumulative drug diffusion.

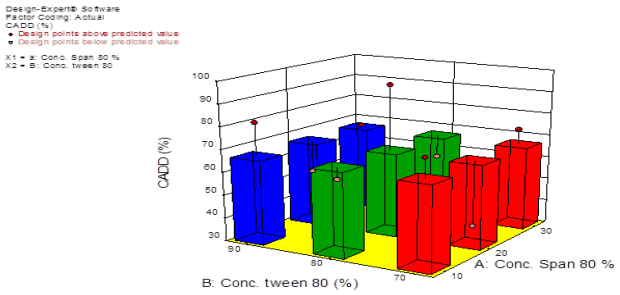

Fig. 9. Response 3D Surface plot for CADD (%).

The response surface plot was generated using Design Expert 10 software and was presented in figures 11.The plot shows that batch F4 gave maximum CADD % compare to other formulation batches.

C. *Morphology*

For an initial characterization of the vesicles, Voriconazole surfactant based formulations were observed microscopically. Optical observation indicated the vesicles were small in size, round in shape and no aggregation was observed in the formulation.

Fig. 10. Optical microscopy of optimized formulation batch 4.

D. *Particle Size Analysis:*

The mean particle size of formulations should be in range of nanometer to micron. The optimized batch of surfactant based vesicles was used for particle size determination. The average particle size of optimized formulation was found to be 251.1 nm, which lies in standard range. Dispersity index was found to be 2.37. Therefore, formulation was found to bepolydisperse in nature.

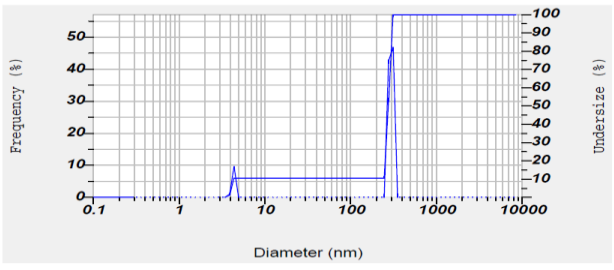

Fig. 11. Graph of particle size of optimized batch (F4).

E. *Zeta Potential Analysis:*

Measurement of Zeta potential is very important parameter in determination of stability of formulation. Highly negative or

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highly positive zeta potential indicates good physical stability. Value > ±20 is essential for effective stability and decrease aggregation. Zeta potential of optimized formulation was found to be -1.6 mV which was not according to standard range.

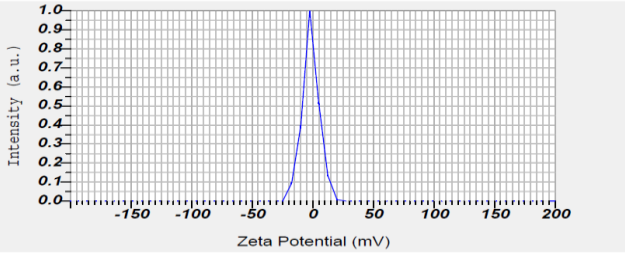

Fig. 12. Graph of zeta potential of optimized batch (F4).

F. Stability Studies:

Optimized formulation was subjected to stability studies as per the literature search. Stability of vesicles is studied by studying aggregation/irregularity and appearance of vesicles over a period of 3 months storage. Table VI shows result of stability studies.

TABLE VI. Observation of stability studies.

Condition for stability	Duration	Appearance	Aggregation
Refrigeration (4˚C -8˚C)	0 day	Clear solution	No
	15 days	Clear solution	No
	30 days	Clear solution	No
	60 days	Clear solution	No
Ambient room temperature (25˚C)	0 day	Clear solution	No
	15 days	Clear solution	No
	30 days	Clear solution	No
	60 days	Clear solution	No

Extent of drug aggregation in refrigerator and at ambient room temperature (25˚C) was significantly low. Hence, the formulation can be refrigerated or stored at room temperature for use.

VII. CONCLUSION

Surfactant based vesicles of Voriconazole containing Span 80 and edge activator (EA) Tween 80 were prepared by ethanol injection method and evaluated. Purity of drug and excipients were confirmed by pre-formulation testing. Purity of drug was confirmed from calibration curve, melting point and analytical method. IR and DSC studies indicated that there were no drug- exciepients interactions. A 32full factorial design was used for formulation of surfactant based vesicles of Voriconazolein which concentration of tween 80 and concentration span 80 were used as 2 factors and experimental trials were performed at all 9 possible combinations which were showed by Design Expert 10 software. The response surface plot was generated using software. This was used to observe the effect of independent variables on the % drug content and corneal permeability. From plot it was observed that formulation batch F4 containing 20:80 (Span 80: Tween 80) showed better corneal permeability (95.83±0.04 %) performed on goat's eye and higher drug content (80.03±0.0152 %) among all formulated batches.

When optimized batch is compared with normal saline pH 7.4 solution it found that newly developed surfactant based formulation gives increase in corneal permeability. In stability study, it was found that extent of drug aggregation upon storage in refrigerator and at ambient room temperature was significantly low. Hence the formulation is stable in nature. Therefore, from the present study it can be concluded that surfactant based ocular drug delivery systems shows good permeability for topical ocular delivery of Voriconazole and it can be used to deliver drugs to the posterior segment of the eye.

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