Investigation Of Formulation Variables In The Design And Development Of Voriconazole Bilosomes

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Abstract:
Voriconazole is a synthetic, second-generation, broad-spectrum triazole derivative of fluconazole that disrupts the cell membrane and stops fungal growth by inhibiting the cytochrome P450 (CYP) dependent enzyme 14-α-sterol demethylase. Bilosomes are non-ionic, amphilic, flexible surfactant delivery vehicles, which contain bile salts to improve oral and skin delivery of drugs at various doses. This study aims to prepare Voriconazole bilosomes as vesicular carriers and assess the effect of different formulation variables such as type and amount of surfactant, amount of cholesterol, amount of bile salts, and sonication time on particle size, entrapment efficiency, and polydispersity index of the prepared bilosomes. The bilosomes were prepared by thin film hydration method and they were optimized using different types of non-ionic surfactants span80 and Tween80 along with different amounts of cholesterol and different sonication times. The objective was to administer voriconazole sustained-release formulation once daily for a whole day to attain an ideal release profile.

Keywords: Fungal infection, Mycosis, Voriconazole, Antifungal activity, Bilosomes, Sustained release.

1. INTRODUCTION:

Voriconazole disrupts the cell membrane and stops fungal development by inhibiting the cytochrome P450 (CYP) - dependent enzyme 14-α-sterol demethylase.
Voriconazole is classified as a Class II drug in the Biopharmaceutics Classification System (BCS). Because of its low solubility, which might affect its bioavailability, voriconazole may have problems with uneven or inadequate absorption. The most widely used method of medicine delivery is oral. The conventional dosage form has several drawbacks, such as variations in the drug's plasma level. These restrictions are overcome by a continuous drug delivery system, which prolongs the duration of the action and reduces drug release to help maintain stable plasma drug concentrations. This study aimed to develop a voriconazole sustained release dosage form to improve effectiveness, reduce dosage frequency, and lessen side effects.

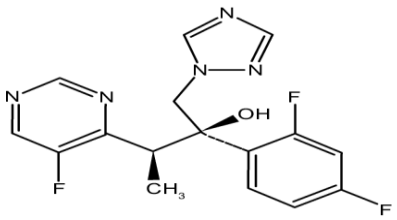


Figure 1: Voriconazole

2. Materials and Methods:

Bile salt Sodium Deoxycholate (SDC), L-α-Phosphatidylcholine, Cholesterol, Tween 80, Span 80, Phosphate Buffer (7.5), Chitosan, Acetic acid, Chloroform.

3. Preparation of Voriconazole Bilosomes:

Take enough sodium deoxycholate, Cholesterol, Tween 80, and Span 80 dissolve it in methanol or chloroform in the round bottom flask. By utilizing a rotary evaporator to remove the organic phase, a thin, dry film of the parts was created. The film was then thoroughly cleaned of any remaining organic phase residue before being rehydrated with 10 ml of phosphate buffer saline (pH 7.4) containing SDC. To prepare Voriconazole-loaded bilosomes, the hydrated dispersion of BS that

resulted was sonicated in a bath sonicator. The finished mixture was monitored for several parameters while being stored in the refrigerator.

Table 1: Formulation table

VZB.Code	Drug (mg)	Sodium deoxycholate (mg)	Cholesterol (mg)	Tween 80 (mg)	Span 80 (mg)	Chloroform (ml)	Phosphate buffer (ml)	Sonication Time (min)
VZB1	200	100	350	10	-	10	5	5
VZB 2	200	100	400	20	-	10	5	5
VZB 3	200	100	450	30	-	10	5	5
VZB 4	200	100	500	40	-	10	5	5
VZB 5	200	100	550	50	-	10	5	5
VZB 6	200	200	600	-	10	10	5	10
VZB 7	200	200	650	-	20	10	5	10
VZB 8	200	200	700	-	30	10	5	10
VZB 9	200	200	750	-	40	10	5	10
VZB10	200	200	800	-	50	10	5	10

Figure 2: Rotary Evaporator

4. Characterization and Evaluation of Voriconazole-Loaded Bilosomes

4.1 Particle size and Poly Dispersity Index (PDI):

Zetasizer-ZS, a photo-correlation spectroscopy technique, was used to measure the particle size and PDI of Voriconazole BS. The mean particle sizes and standard deviations based on the PDI were then shown. To verify that there hasn't been any particle aggregation, more measurements were conducted.

4.2 Entrapment Efficiency:

The %EE of Voriconazole in the bilosomes might be ascertained by measuring un-entrapped Voriconazole in the dispersion media. One millilitre of bilosomes was put in a centrifugation tube and spun at 16,000 rpm for an hour at 4 °C using a cooling centrifuge (all formulas were separated from free drug using a cold centrifuge; only three formulas were repeated using amicon tubes; the results indicated a correlation between the two methods, so continue with the cold centrifuge). A UV-VIS spectrophotometer set to 237 nm was used to measure the drug concentration following the separation and PBS dilution of the resultant supernatant. As a result, %EE was determined using the subsequent equation.

$$EE\% = \frac{Total\ amount\ of\ drug - amount\ of\ free\ drug}{\text{Total amount of drug}} \times 100$$

4.3 Microscopic examination of Voriconazole-loaded Bilosomes:

To identify the morphology of the Voriconazole-loaded bilosomes, one drop of Bilosomal dispersion was taken on the glass slide and observed under a projection microscope with 40X magnification and 100X.

4.4 Transmission electron microscope (TEM):
The prepared Voriconazole-loaded bilosomes form was ascertained using a high-resolution transmission electron microscope (TEM). A drop of the produced formulae was collected, diluted appropriately with distilled water, and placed on carbon-coated copper grids to dry at room temperature for ten minutes. Afterward, the sample was inspected to determine its shape.

4.5 Differential scanning calorimetry (DSC):
Using a differential scanning calorimeter fitted with an intercooler, the thermal behaviour of voriconazole and its compatibility with other formulation constituents were assessed. Samples weighing 2-4 mg were precisely weighed, put in aluminum pans, and heated in a nitrogen purging gas environment at a rate of 10°C per minute between 30 and 300°C.

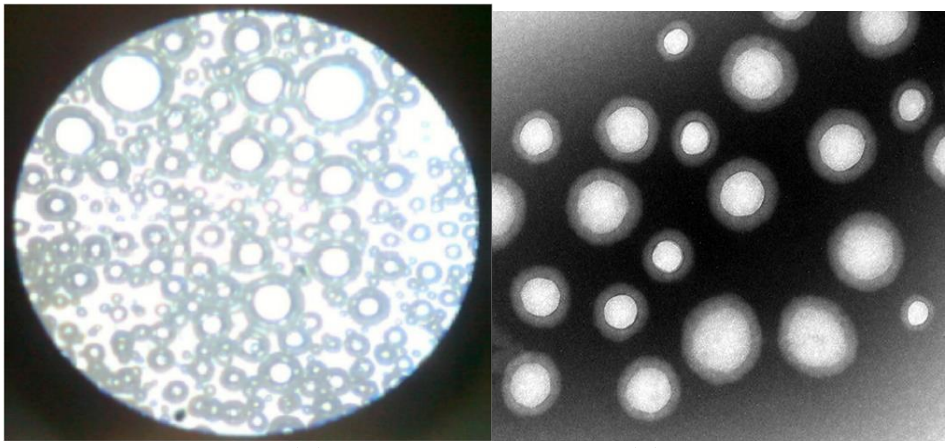
***4.6 In vitro* drug release:**
In vitro drug release was performed for the selected Bilosomal formulation (VZB10) in comparison with that of pure drug suspension. Two millilitres was taken from the selected formula VZB10 and pure drug suspension they were poured in dialysis bags (which were soaked overnight in the release media). Then the dialysis bags were placed in type two dissolution apparatus (Paddle type) at 37±0.2^{0}C and rotation speed 50 rpm, the release media was 250 ml PBS solution (containing 0.75% w/v SLS) to achieve sink condition.
At predetermined time (1, 2, 4, 6, 8, 10,12, 14, 16, 18, 20, 22 and 24 hours) three millilitres samples were withdrawn and replaced by fresh PBS solution to maintain sink condition. The cumulative amount release of Voriconazole was measured by UV/VIS spectrophotometer at 236 nm.

5. Variables affecting formulation
Different types of surfactants, different surfactant: cholesterol ratios, different concentrations of bile salts along different sonication times were used for the preparation of Voriconazole-loaded Bilosomes.

5.1 The effect of a different surfactant type was studied in the formulas VZB1, VZB2, VZB3, VZB4, and VZB5. In each of these formulations, the amount of Voriconazole (200 mg) the quantity of SDC (200 mg), and the sonication time of 5 min were kept constant.

5.2 The effect of the surfactant: cholesterol ratio on the formulations of Voriconazole-loaded bilosomes was evaluated in formulas VZB6, VZB7, VZB8, VZB9, and VZB10. In each of these formulas, the concentration of the drug (200 mg), bile salt (200 mg), and sonication time (10 min) were all kept constant.

6. Results and Discussion
6.1 Optical microscope morphology:
Results of the light microscope for formula (VZB10) under oil immersion at 100 X and 40X shown in (Figure 1) indicate the formation of vesicles.



(A) (B)
Figure 3: optical microscopic image A) at 40 X B) at 100 X

6.2 TEM: TEM was done for some of the Voriconazole-BS formulations and the results showed the presence of bilayer vesicles with different particle sizes as shown in (Figure 3).

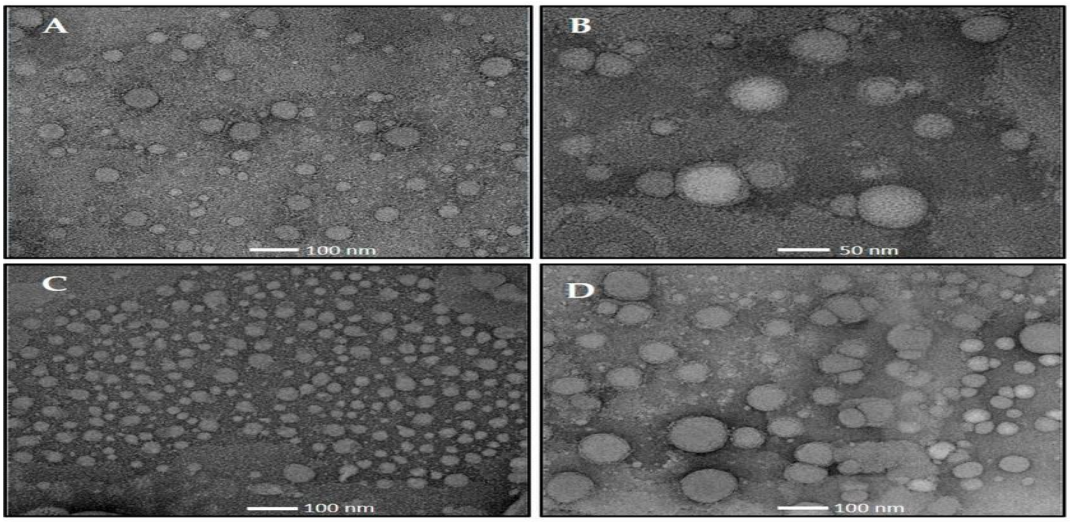
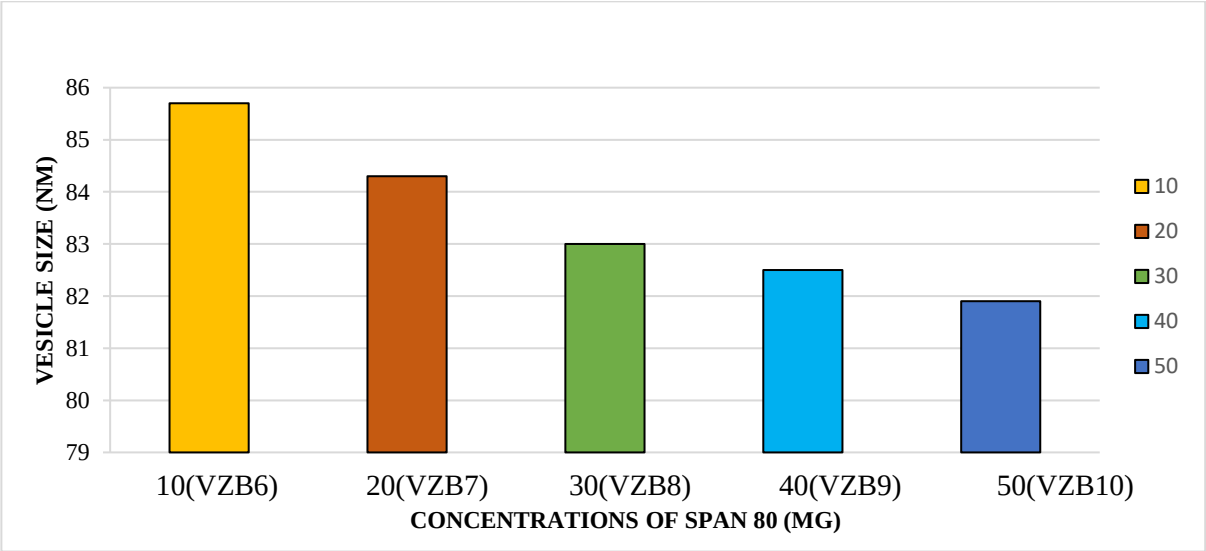
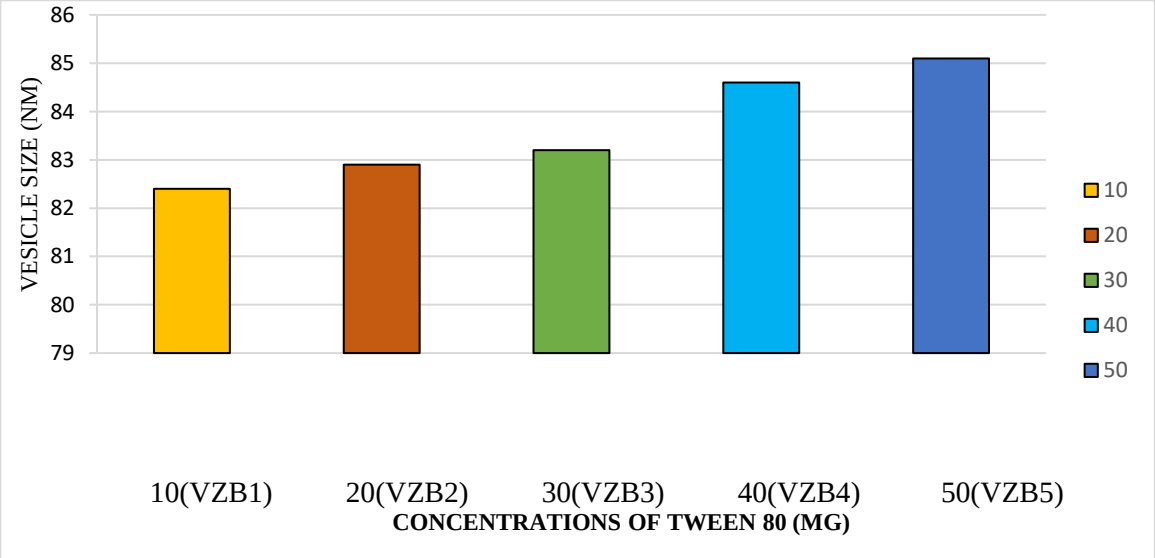


Figure 4: TEM images from different Voriconazole-loaded Bilosomes

6.3 Vesicle size: Table (2) shows the vesicle size of the Voriconazole-loaded bilosomes formulations which were found in the range of 81.9 ±0.74 - 85.7±1.72.



(A)



(B)

Figure 5: Effect of type of surfactant and its concentration on vesicle size (A)Effect of Span 80 (B) Effect of Tween 80

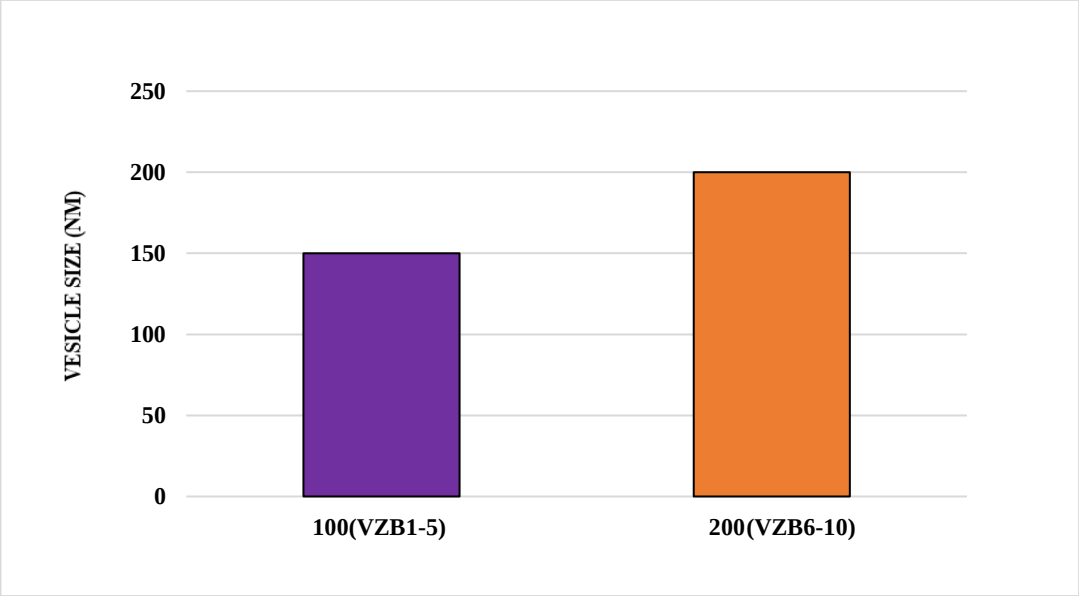


Figure 6: Effect of SDC on vesicle size

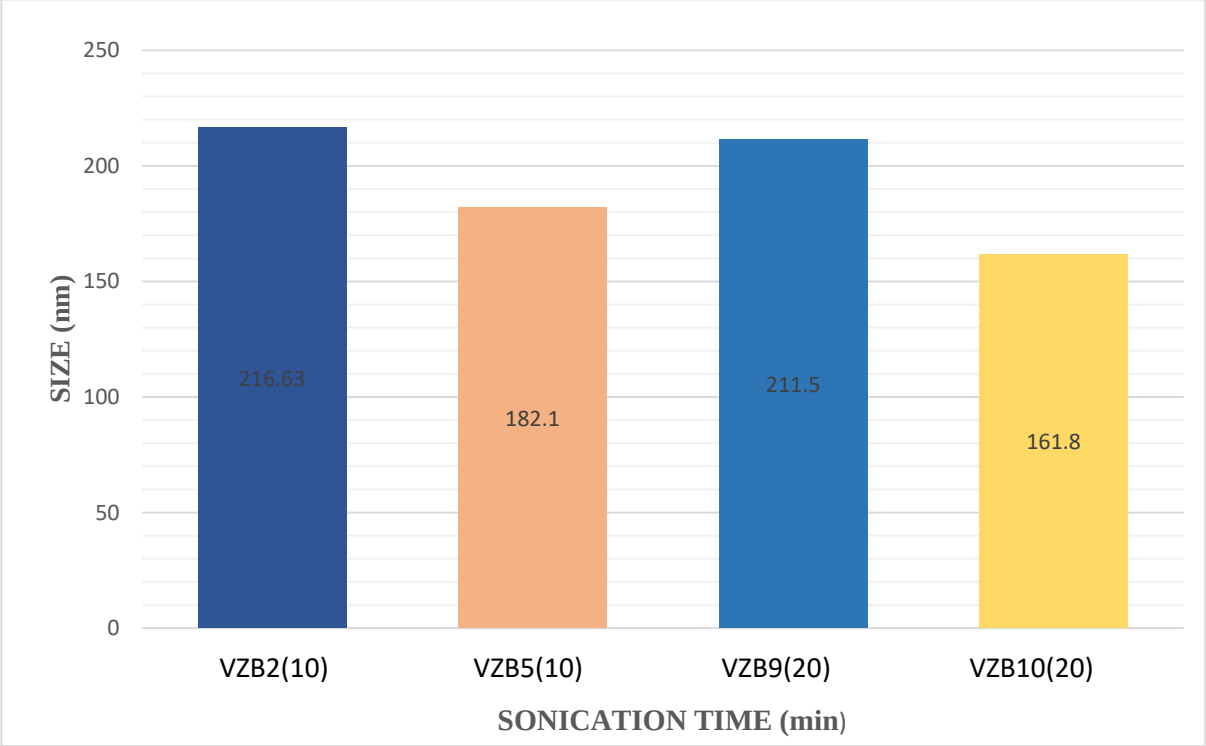


Figure 7: Effect of Sonication Time on Vesicle Size

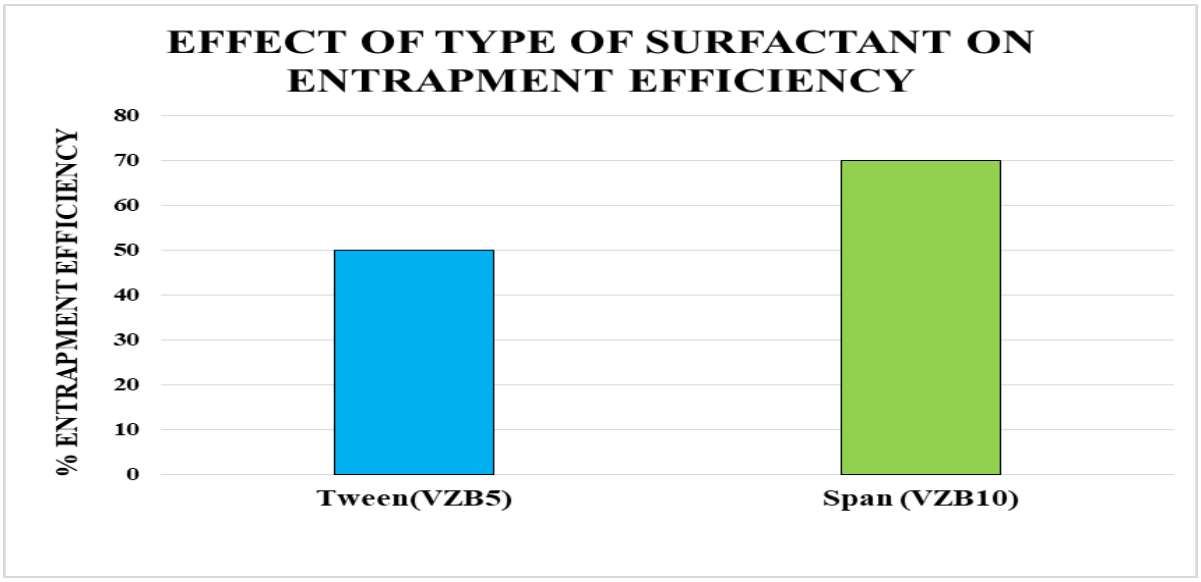
Formulation Code	Particle Size (nm)	PDI (Mw)	Entrapment Efficiency (%)
VZB1	82.4	0.3±0.04	45.46±0.1%
VZB2	82.9	0.26±0.13	58.58±0.17%
VZB3	83.2	0.28±0.025	65.1±0.46%
VZB4	84.6	0.19±0.03	66.36±0.80%
VZB5	95.1	0.25±0.13	65.13±1.13%
VZB6	85.7	0.36±0.035	65.2±1.10%
VZB7	84.3	0.26±0.058	58.42±0.39%
VZB8	83.2	0.33±0.3	71±0.42%
VZB9	82.5	0.19±0.081	60.13±0.78%
VZB10	81.9	0.38±0.15	80.13±0.64%

Table 2. Mean Particle Size (PS), Polydispersity Index (PDI) and Entrapment Efficiency of Different Voriconazole BS formulations.

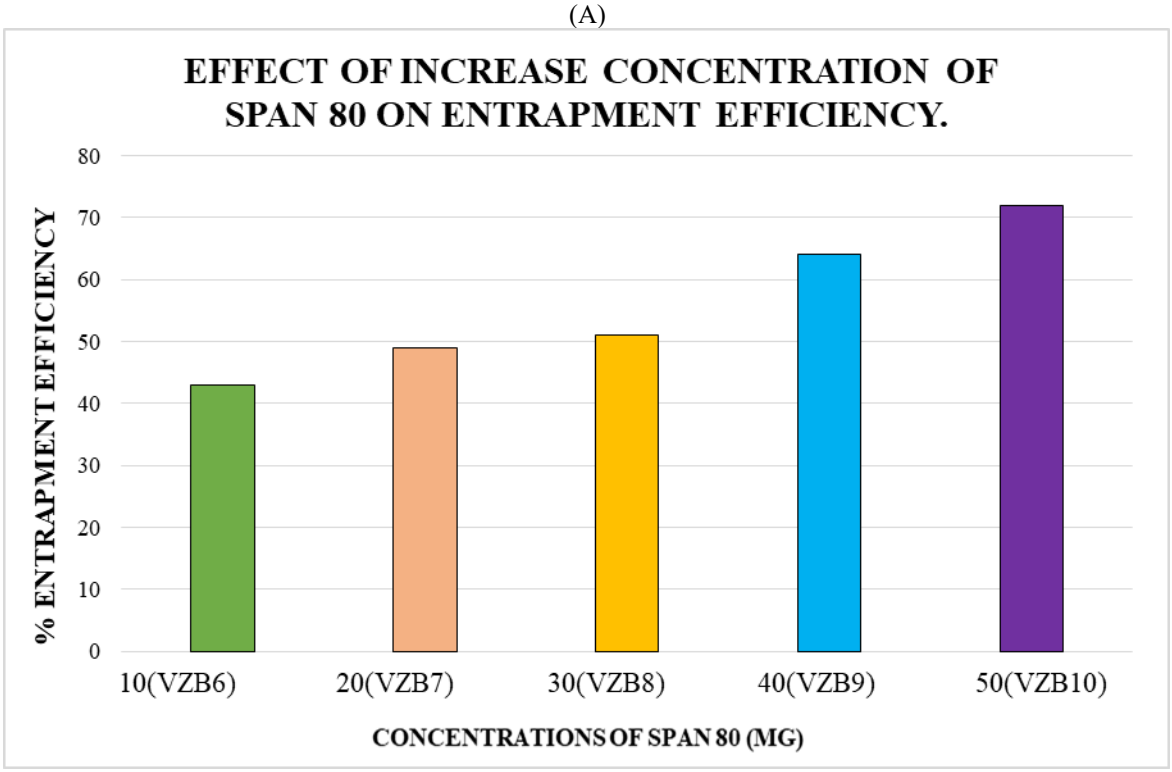
6.4 Entrapment Efficiency:

The impact of the length of the alkyl chain on the BS Vesicles can be explained by the fact that the BS created with Span 80 (VZB10) generally displayed greater entrapment efficiency (80%) than those formulated with the tween80 (VZB5) and span80 (VZB6) that showed entrapment efficiency 65% and 77% respectively, as seen in the Figure ure7.

By increasing the span 80 concentration the entrapment efficiency was increased significantly from 65% to 80% due to the fact that increase span 80 concentration reduces the interfacial energy as well as increase the viscosity of the dispersion, which is prime to avoid the leak of drug from vesicles.



(A)



(B)

Figure 8: (A) Effect of type of surfactant on Entrapment Efficiency (B) Effect of concentration of surfactant on Entrapment efficiency.

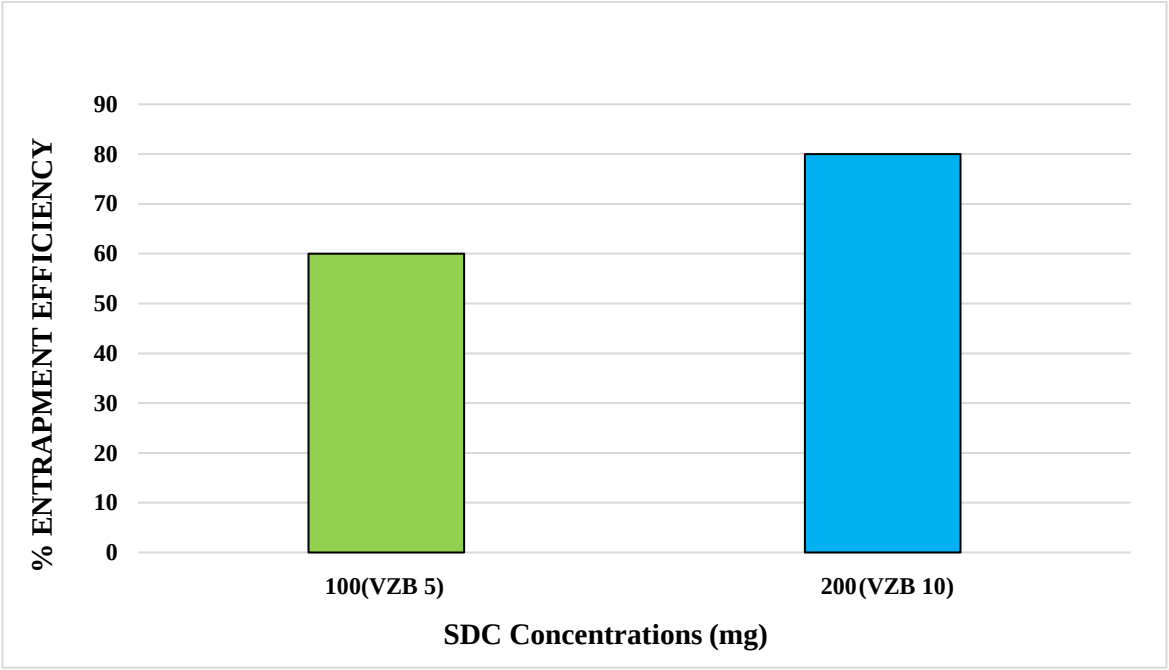


Figure 9: Effect of concentration of SDC on Entrapment Efficiency

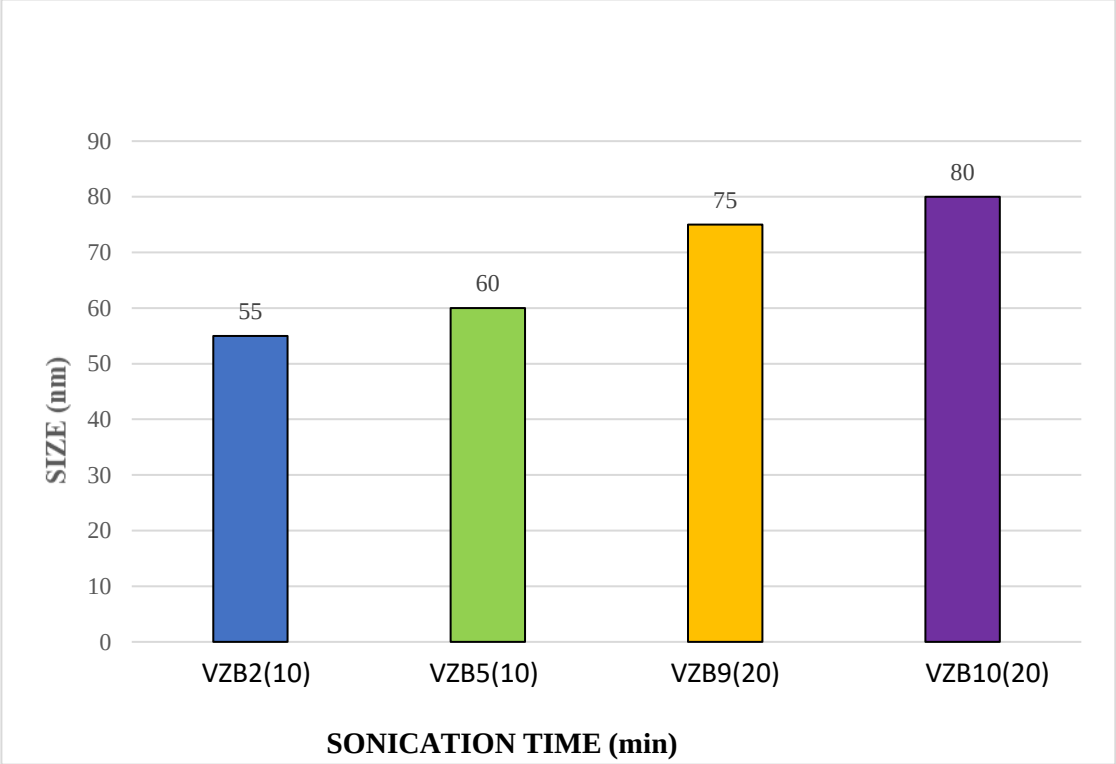


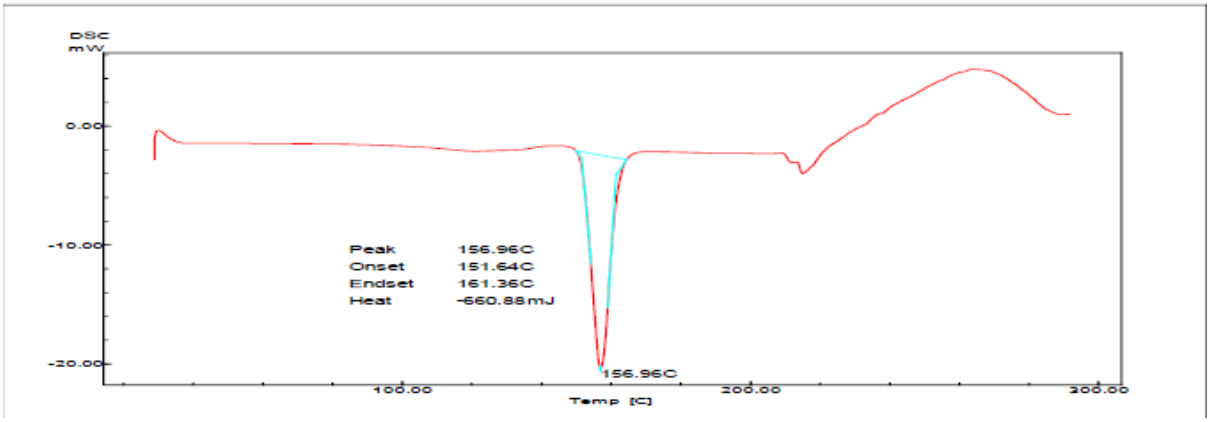
Figure 10: Effect of sonication time on Entrapment Efficiency

6.5 Poly Dispersity Index (PDI):

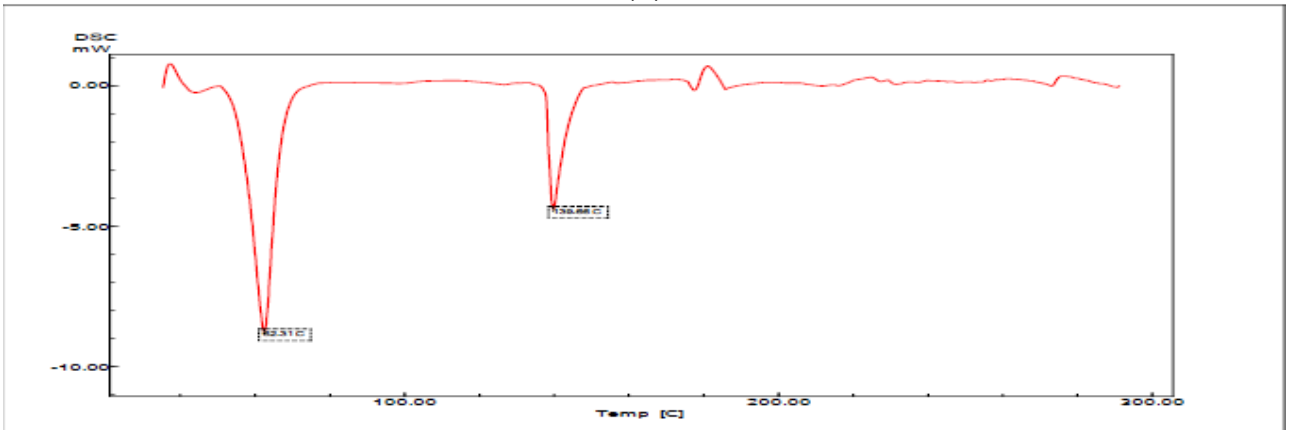
All Voriconazole-BS formulations showed PDI between 0.1-0.4 which indicate homogeneous Formulations. PDI values (Table2) of ≤0.3 indicates homogenous monodispersed formulation with good stability and uniformity in droplet size distribution upon dilution.

6.6 Differential Scanning Calorimetry:

DSC thermo gram of pure drug, physical mixture of drug and other components and lyophilized Bilosome formulation (VZB10) are shown in Figure 11.



(A)



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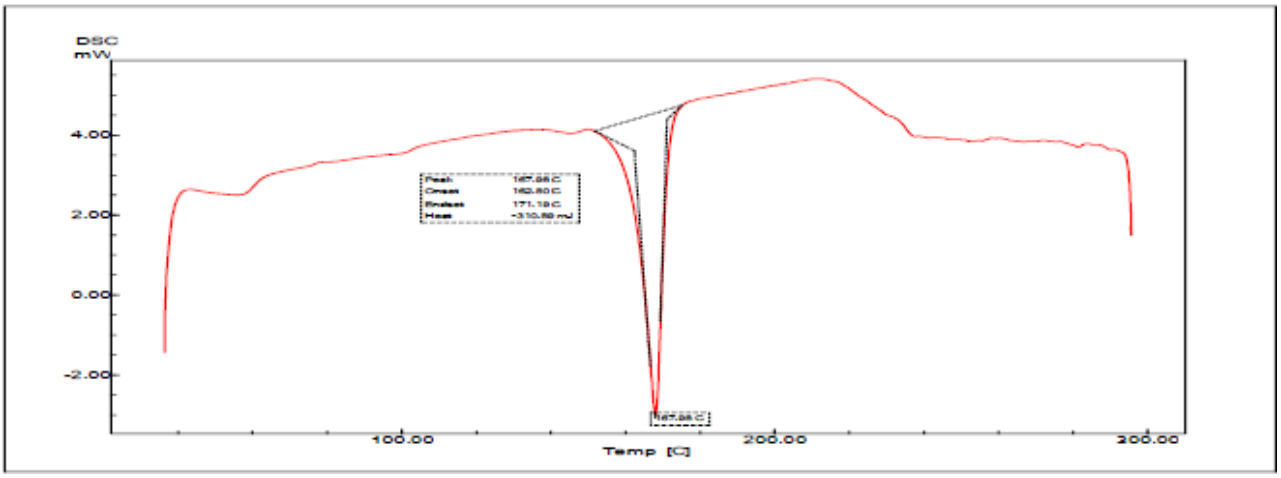

(C)

Figure 11. DSC thermo gram (A) pure drug (B) physical mixture (C) optimum formula VZB10.

6.7 *In-vitro* release:

The *in vitro* release profile of Voriconazole from the VZB10 BS formulation revealed that 96.61% of the drug released. although it did so in a biphasic manner, releasing the majority of its dose in the first two hours (25.43%), followed by a continuous release of the drug (96.61).

The sustained release of voriconazole from the optimized bilosomes was caused by the high affinity of voriconazole for the hydrophobic counterpart of the vesicles, while the initial burst release of voriconazole from the bilosomes was caused by the desorption of voriconazole from the surface of the bilosome vesicle in the first two hours.

Voriconazole would be quickly released in the beginning and then slowly released, allowing the patient to carry on the other hand, an 8-hour period of 41.13% drug release was observed in the in vitro release profile for Vorionazole solution. When compared to pure drug of voriconazole the bilosomes demonstrated a considerably greater release of the VZB10.

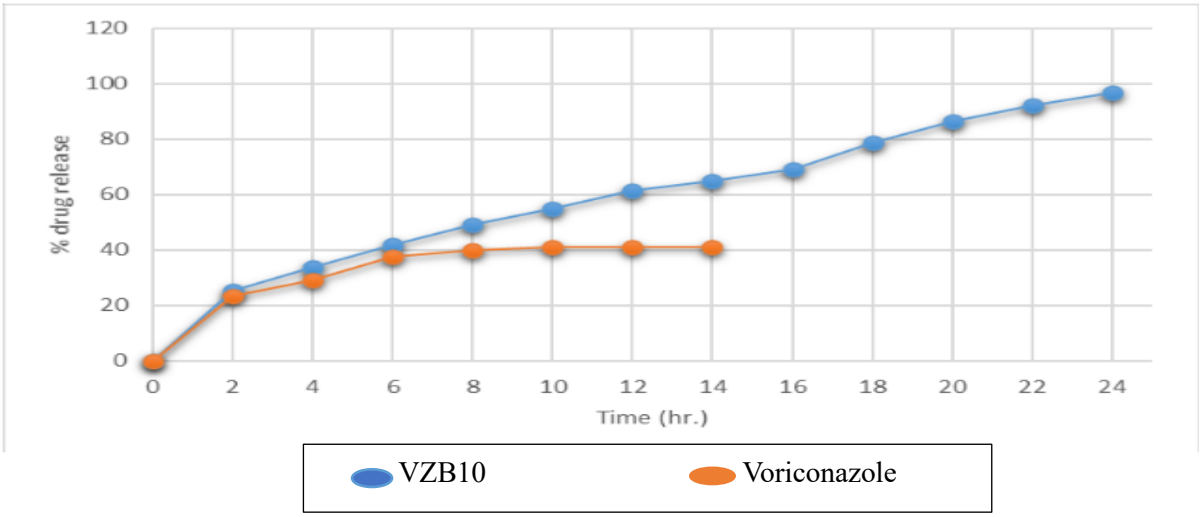

Figure 12. *In Vitro* release profile of optimum bilosomes formula VZB10 in comparison with pure drug of voriconazole .

7. Conclusion:

The present research work was designed to prepare voriconazole bilosomes for topical delivery for antifungal activity. In this study, Voriconazole Bilosomes were developed to sustain its action, leading to better patient compliance and reducing the incidence of adverse side effects.

8. Acknowledgements:

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9. References:

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