

DESIGN AND INVITRO EVALUATION OF VORICONAZOLE NIOSOMES

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Received: 12 Apr 2013, Revised and Accepted: 29 May 2013

ABSTRACT

Objective: The aim of this present work is to formulate Voriconazole niosomes by two different methods in various ratios of surfactant span 80.

Methods: Voriconazole niosomes prepared by hand shaking and ether injection method by using span 80 and cholesterol from which the best formulation was selected and characterize in terms of vesicles size distribution, entrapment efficiency and invitro release studies. Interaction of drug and the different ingredients in the niosomes were studied by FTIR spectra and found to be compatible.

Results: The niosomes size range of prepared formulation 0.5-5 μ and 0.5-2.5 μ by hand shaking method and ether injection method respectively. The entrapment efficiency of Voriconazole niosomes was determined by separating the entrapped drug by dialyzing method. The entrapment efficiency of the drug was found to be more than 84.53% in case of niosomes prepared by hand shaking process. The in vitro release profile of drug from niosomes was studied in 0.1 N HCL using dialyzing method and result indicated 70.06% drug release for the formulation prepared by hand shaking method and It took an extended period of 24 hours of drug release.

Conclusion: In vitro release of Voriconazole from niosomes was very slow when compared to the release from pure Voriconazole solution.

Keywords: Diethyl ether, Ether injection method (ES), Hand shaking method (HS), Span 80.

INTRODUCTION

Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and low permeability through epithelium and connective tissue keeps the drug localized at the site of administration. Localization of drug action results in enhancement of efficacy or potency of the drug and at the same time reduces its systemic toxic effects. The niosomes are targeted to reticuloendothelial system and to other organs, other than reticuloendothelial system in the body by use of antibodies, immunoglobulins. Many cells possess the intrinsic ability to recognize and bind to the drug carrier system. Niosomes can provide relatively constant and sustained blood stream levels of drug concentration. Niosomes can be injected into circulation and thus can serve as an intravascular drug depot. Sustained release action of niosomes could be applied to drugs with low therapeutic index and low water solubility, since these could be maintained in the circulation via niosomal encapsulation.

Voriconazole is an antifungal drug, used in the treatment of fungal infections including fusarium spp. And scedosporium apiospermum - asexual form of pseudoallescheria boydii, was used as a model drug

to be encapsulated as niosomal drug delivery system. Voriconazole has a short biological half life, which necessitates multiple daily dosing and hence a novel delivery system such as niosomes, can be used to encapsulate the drug so that it maintain a therapeutic plasma concentration for a longer period of time, thereby increasing the bioavailability of the drug. Hence this niosomal delivery may reduce the frequency of dosing intervals and may improve patient compliance.

MATERIALS AND METHODS

a) Preparation of voriconazole niosomes by hand shaking method [1-2]

Cholesterol and span 80 were taken in specified ratios of (1:1, 1:2&1:3) and transferred in to a clean round bottom flask (table. 1). Then the lipid mixture was dissolved in 10 ml of diethyl ether. The flask was continuously vortexed to form a thin film along the sides of the flask. An appropriate amount of Voriconazole was dissolved in phosphate buffer saline (PBS) pH.7.4. This was poured into added to the thin film and vortexed continuously for a period of 30 min at room temperature.

Table 1: Composition of the Voriconazole Niosomal formulations

Formulation Code	Drug	Span 80	Cholesterol	Diethyl ether
HS 1	50 mg	50 mg	50 mg	10 ml
HS 2	50 mg	100 mg	50 mg	10 ml
HS 3	50 mg	150 mg	50 mg	10 ml

b) Preparation of voriconazole niosomes by ether injection method [3, 4]

Cholesterol and span 80 were taken in prescribed ratio (1:1, 1:2 &1:3) in a 50 ml beaker (table 2). The mixture was dissolved in

diethyl ether and the solution was slowly injected into a beaker containing Voriconazole in phosphate buffer saline (PBS) pH 7.4. The temperature maintained during the injection was 40-60 $^{\circ}$ c. The differences in temperature between phases cause rapid vaporization of ether resulting in spontaneous vesiculation.

Table 2: Composition of the Voriconazole Niosomes Formulations

Formulation Code	Drug	Span 80	Cholesterol	Diethyl ether
ES 1	50 mg	50 mg	50 mg	10 ml
ES 2	50 mg	100 mg	50 mg	10 ml
ES 3	50 mg	150 mg	50 mg	10 ml

c) Evaluation of different batches of prepared voriconazole niosomes formulation

i. Morphology

The prepared niosomal vesicles were mostly round in shape and some vesicles are slightly elongated. The vesicles are slightly yellow in colour. Individual vesicles could be seen as shown in the photograph, more over these vesicles were viscous and it can visualize under microscope after dilution with water.

ii. Vesicles size determination

1. By optical microscopy [5-7]

Take a one drop of prepared niosomes (diluted with water if needed) in a clean glass slide. Then it is placed on the stage of the optical microscope. First it was viewed under low power then changed to high power and the vesicles were counted up to 100 numbers.

2. By Scanning Electron microscopy (SEM) [8]

The sizes of the vesicles are to be measure by scanning electron microscopy (HITACHI S - 150). Take the small amount of sample of niosomes suspension in cover slip on the specimen stub. Coat it with carbon and then with gold vapor using Hitachi vacuum evaporator, model HITACHI S 5 GB. The samples are examine under scanning electron microscope, which is operate at 15 kilovolts and then photograph it.

iii. Percentage Encapsulation of drug

The total amount of drug will not be encapsulated into the vesicle (niosomal vesicle) they may be present in free form (unencapsulated). So for the determination of percentage drug entrapment in the vesicle, it is necessary to separate untrapped drug in formulation.

The separation of untrapped drug can be done by the following techniques

- I. Exhaustive Dialysis.
- II. Separation by gel filtration.
- III. Centrifugation (7000 X G for 30 min.)
- IV. Ultra Centrifugation (150000 X G for 1.5 H).

Voriconazole encapsulated niosomes were separated from untrapped drug by dialysis method¹⁰ for 24 hrs. The formulation were transferred into a standard flask, then lysed with 1ml of 2.5% SLS solution, then incubated at $37^{\circ} \pm 1^{\circ}$ C for 2 hrs. Then it is filtered through whatman filter paper. Filtrate of 1 ml was diluted to 10 ml with PBS and absorbance of the resulting solution was measured spectrophotometrically at 255 nm. [9]

iv. Determination of percentage of drug encapsulated in the niosomes

This was carried out to find out the percentage of drugs encapsulated in the niosomes by using the following formula,

$$\text{Percentage of Drug Encapsulated} = \frac{\text{Amount of encapsulated drug in niosomes (mgs)} \times 100}{\text{Amount of drug used (mgs)}}$$

v. Osmotic shock [10]

Effect of osmotic shock was determined by measuring the change in vesicles size followed by incubating the vesicular suspension in media of different tonicity such as a hypertonic media with 1.5% w/v NaCl solution, normal saline with 0.9% w/v NaCl, hypotonic media with 0.5% w/v NaCl and PBS. The vesicular suspension was incubated in these media for 3 h following which the change in vesicle size was measured.

vi. Drug leakage studies from vesicles [11]

Vesicle stability with respect to drug leakage and drug degradation upon storage was studied at refrigeration (4° C), room temperature (25° C) and high temperature (37° C) for a period of one month on niosomes samples containing a known amount of Voriconazole, contained in light resistant containers. Samples were withdrawn at weekly intervals, and entrapment efficiency was determined.

vii. In Vitro Release Pattern of Voriconazole Niosomal Formulations [12]

The niosomal preparation was taken in a dialysis tube, which acts as a donor compartment. Dialysis tube was placed in a beaker containing 250 ml of phosphate buffer saline of pH 7.4, which acts as a receptor compartment. The temperature of receptor medium maintained at $37^{\circ} \pm 1^{\circ}$ c and medium agitated at a moderate speed using magnetic stirrer. 1 ml aliquots of sample was taken and made up to 10 ml with PBS. 1 ml of diffusion medium was replaced after every withdrawal so that the volume of the diffusion medium was maintained. The collected samples were analysed at 255nm.

RESULTS AND DISCUSSION

Niosomes of Voriconazole were prepared by hand shaking method and ether injection method using cholesterol and various ratios of Span 80.

Niosomes were evaluated for morphology, vesicle size determination, and percentage of drug encapsulation, drug leakage studies from vesicles, osmotic shock and in vitro release profile.

A) Compatibility studies

The IR Spectrum of pure Voriconazole drug was compared with the IR spectrum of physical mixture of drug and excipients. (Fig.1-4)

There is no appearance or disappearance of any characteristics peaks. This shows that there is no interaction between the drug and excipients used in the vesicles preparation.

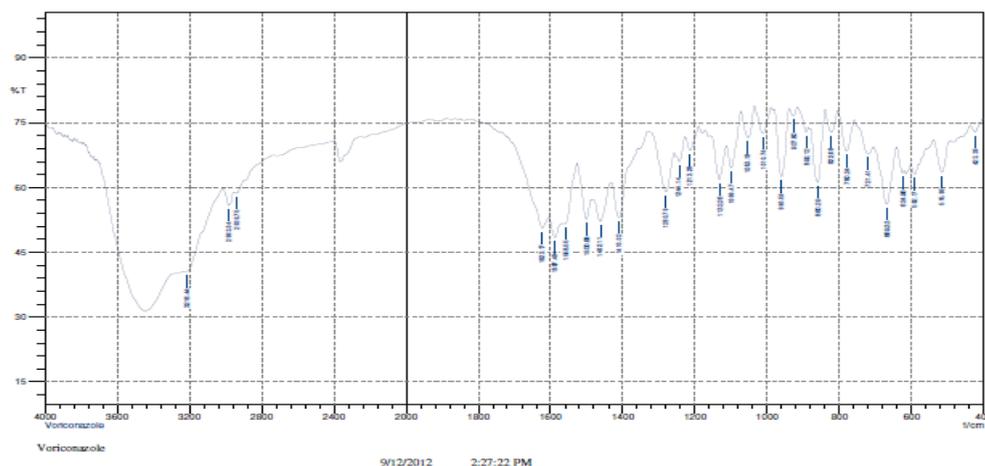


Fig. 1: FTIR spectra of voriconazole

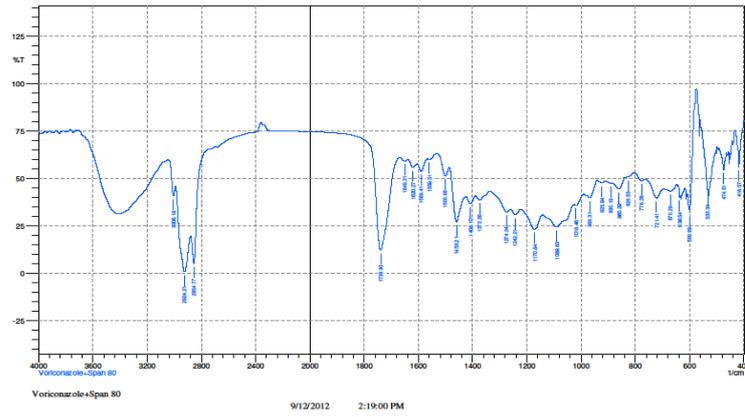


Fig. 2: FTIR spectra of Voriconazole and span 80

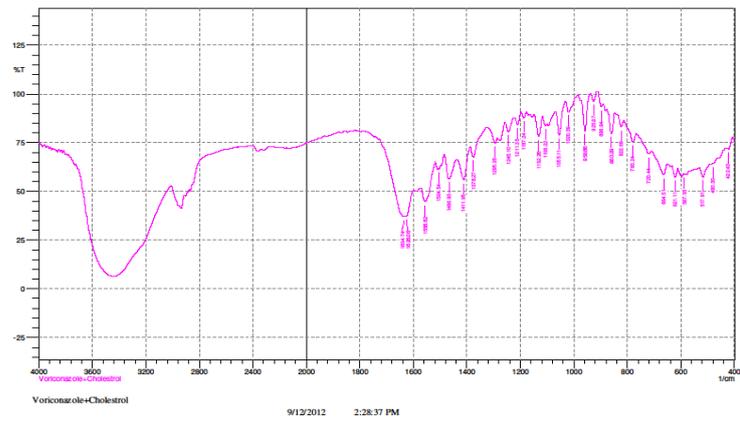


Fig. 3: FTIR spectra of Voriconazole and Cholesterol

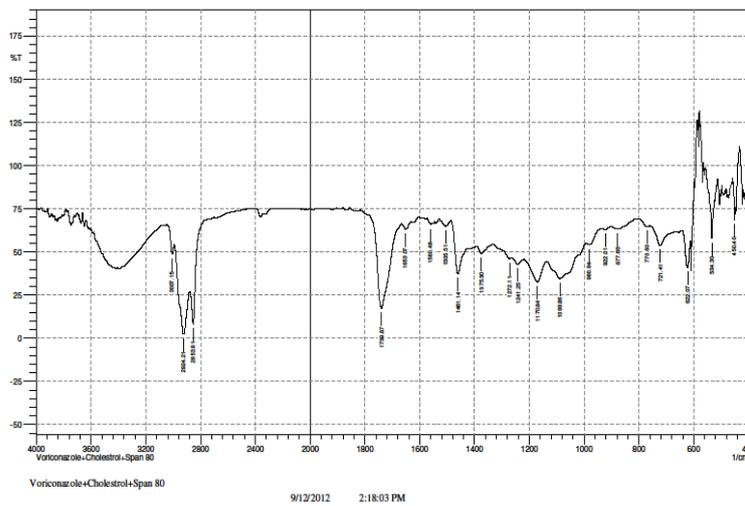


Fig. 4: FTIR spectra of Voriconazole, Cholesterol and span 80

B) Vesicle size determination**i) By optical microscopy**

The formed vesicles from all batches of formulation were viewed under the high power objective in the optical microscope. It was also observed that niosomes prepared by hand shaking method was comparatively

larger (fig-5) of size range (0.1-5 μ m) with an average size of 3.5 μ m than niosomes prepared by ether injection method of size range (0.5-2.5 μ m) (table 3) with an average size 1.5 μ m (fig-6). The reason for reduced size of the vesicles may be because in ether injection method, Span 80 and cholesterol mixture is injected in to drug solution by forcing it through a needle orifice, so that smaller vesicles were formed.

Table 3: Size Distribution of Niosomes by Hand Shaking Method, Ether Injection Method

Number of Niosomes	Size range (μ m) (Hand Shaking Method)			Size range (μ m) (Ether Injection Method)		
	Below 0.1	0.1-5	Above 5	Below 0.5	0.5-2.5	Above 2.5
1:1:1 Ratio	18-20	70-72	13-17	18-20	65-70	3-6
2:1:1 Ratio	16-18	72-75	6-7	13-15	68-74	82-86
3:1:1 Ratio	8-9	85-87	5-9	6-8	82-86	4-5



Fig. 5: Optical Microscopic view of Voriconazole loaded Niosomes prepared by Hand Shaking Method for Formulation HS 3 (20 x 40X)

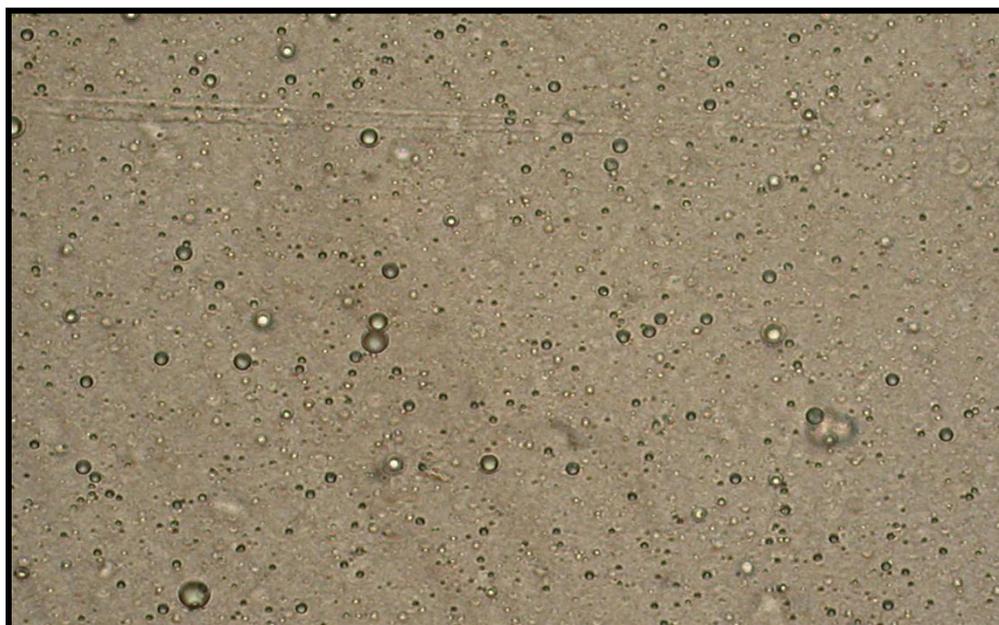


Fig. 6: Optical Microscopic view of Voriconazole loaded Niosomes prepared by Ether Injection Method for Formulation ES 3 (20 x 40X)

ii) By scanning electron microscopy (SEM)

The prepared niosomes vesicles sizes were performed by SEM. The size range was observed to be 0.5-5 μ m for niosomes prepared by hand shaking method and 0.5-2.5 μ m in ether injection method. This is shown in the photographs. (Fig-7, 8).

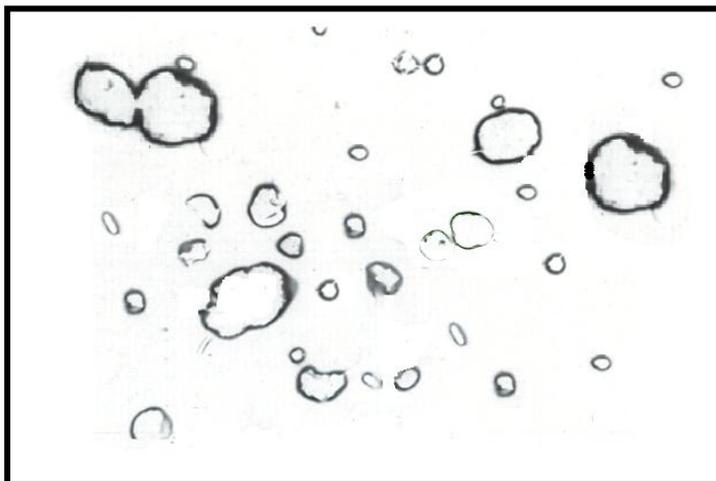


Fig. 7: Scanning Electron Microscopic view of Voriconazole loaded Niosomes by Hand Shaking Method For formulation HS 3 (400 X)

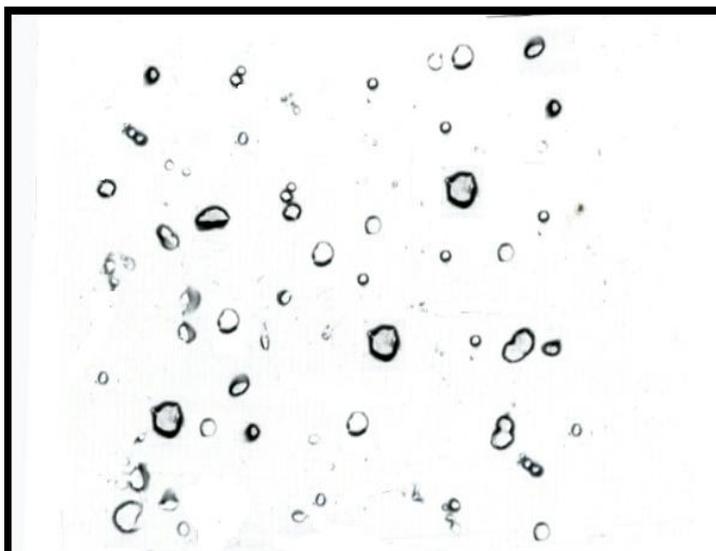


Fig. 8: Scanning Electron Microscopic view of Voriconazole loaded Niosomes by Ether Injection Method For formulation ES 3 (300 X)

C) Osmotic shock

Effect of osmotic shock was studied by incubating the vesicular suspension in media of different tonicity for a period of 3 h. The vesicular suspension incubated with hypertonic media (1.5 % w/v NaCl) for a 3 h, shrunk completely (Tab-4). This was observed with

all the formulations. Similarly, incubation of vesicular suspension in a hypotonic media (0.5 % w/v NaCl) recorded an increase in their vesicle size (Tab-4). This was a significant increase as compared to the vesicular dispersion in PBS (pH 7.4) (Tab-4). However, no significant change in vesicle size was observed with vesicles incubated in normal saline (0.9 % w/v NaCl).

Table 4: Average vesicle size for Voriconazole encapsulated niosomal formulation

Formulation Code	Average vesicle size (μ m) after incubation with			
	PBS (pH 7.4)	1.5 % w/v NaCl	0.9 % w/v NaCl	0.5 % w/v NaCl
HS 1	3.84	Shrunked	3.96	6.24
HS 2	4.21	Shrunked	4.31	6.58
HS 3	4.54	Shrunked	4.62	6.97
ES 1	0.61	Shrunked	0.64	0.92
ES 2	0.73	Shrunked	0.74	1.51
ES 3	0.75	Shrunked	0.77	1.68

D) Drug leakage studies from vesicles

The bar diagram between percent drug remaining in niosomes at 7, 14, 21, & 28 days at refrigeration temperature, room temperature and high temperature has been represented in (Tab 5). Niosomes have shown a fairly high retention of the drug inside the vesicles at a refrigeration temperature up to a period of one month ($\approx 90\%$).

While, storage at room temperature lead to a substantial loss ($\approx 20\%$) and storage at high temperature lead to a substantial loss ($\approx 40\%$) of the drug from the niosomes at the end of one-month period.

The drug leakage at elevated temperature may be related to the degradation of lipids in bilayers resulting in defects in membrane packing making them leaky.

Table 5: Percentage of drug retention in Voriconazole niosomal formulation

Formulation Code	Percentage of drug retention in niosomes											
	Refrigeration Temp. ($4^{\circ} \pm 1$ C)				Room Temp ($25^{\circ} \pm 1$ C)				High Temp. ($37^{\circ} \pm 1$ C)			
	7 Days	14	21	28	7 Days	14	21	28	7 Days	14	21	28
HS1	100	95	90	86	99	88	84	77	94	85	73	69
HS2	100	96	95	87	99	90	84	79	95	84	75	70
HS3	100	97	95	90	100	94	87	80	95	85	76	70
ES1	100	95	93	85	98	93	84	77	93	84	73	68
ES2	100	97	94	86	99	95	85	78	94	85	74	69
ES3	100	98	95	90	100	95	88	80	96	86	75	72

E) Percentage of Drug Encapsulation

The percentages of drug encapsulated in niosomes are given in (Tab-6). Percentage of drug encapsulation varies when the Span 80 proportion was varied. This explained that encapsulation was increased with increase in Span 80 content while cholesterol content was maintained at constant value. Niosomes prepared by hand shaking method have good encapsulation efficiency. This may be due to the good vortexing and more over the vesicles were larger. In the case of ether injection method, the vesicles were smaller; this may cause a decrease in encapsulation. A good percentage of drug encapsulation was achieved from batches HS3& ES 3. Least percentage of drug encapsulation was achieved from batches HS 1 and ES 1.

F) In vitro drug release studies

In vitro drug release was studied for all the batches of niosomes. The studies were performed up to 24 h for all the batches. The cumulative % release of Voriconazole from various formulations by hand shaking method at 24 h was found to be 65.51%, 74.91% and 84.53% for HS 1, HS 2 and HS 3 respectively. The above result indicates that though the entrapment efficiency was increased with HS 2 and HS 3 formulation, the release was little fast. But in the HS 1 formulation though

entrapment was less, the drug was release for a longer duration. Niosomes prepared by ether injection method showed little less entrapment efficiency than niosomes prepared by hand shaking method. The release pattern of batch ES 1 and ES 2 did not show so much difference. This may be due to the formulation of similar size vesicles in both the batches. But in the case of batch ES 3, the release rate is more. An almost constant drug release was observed in all formulations indicating zero order release pattern (fig-9-11) (Tab-7-9). This is indicated by the linearity in regression value when plotted with cumulative percentage release vs time curve.

Table 6: Encapsulation efficiency for Voriconazole Niosomal Formulations

Formulation Code	Amount of drug used in (mgs)	Percentage of drug encapsulated
HS 1	50 mg	56
HS 2	50 mg	65
HS 3	50 mg	84
ES 1	50 mg	45
ES 2	50 mg	54
ES 3	50 mg	70

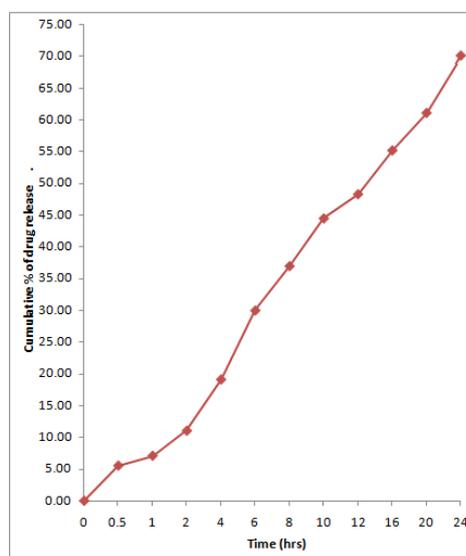


Fig. 9: In vitro release profile of Voriconazole Niosomes for Formulation ES-3

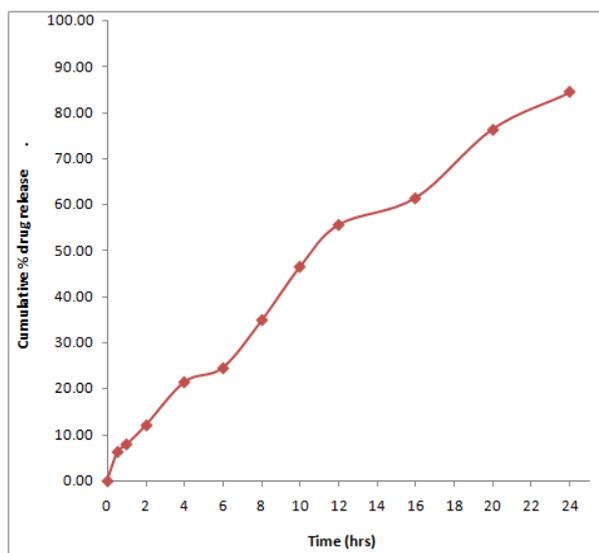


Fig. 10: In vitro release profile of Voriconazole Niosomes for Formulation HS-3

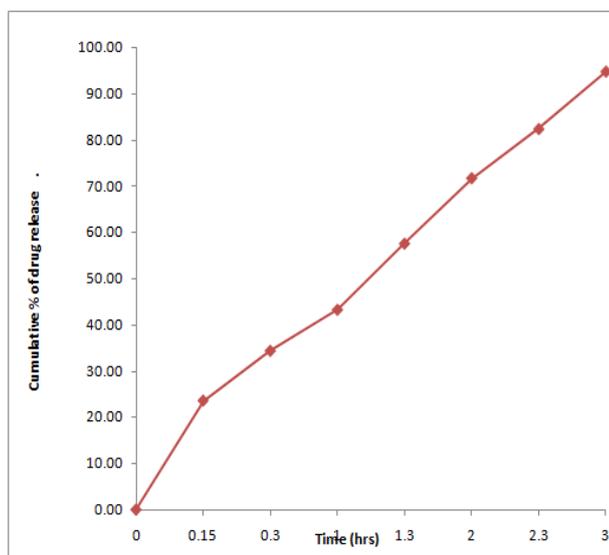


Fig. 11: In vitro release profile of pure Voriconazole drug

CONCLUSION

Stable Voriconazole loaded Niosomes can be prepared by hand shaking method and ether injection method with Span 80 and cholesterol in the ratio of 1:1, 2:1, and 3:1. Most of the vesicles are spherical in shape, the size range of the vesicles, fall in the narrow size range of 0.5-5 μ and 0.5-2.5 μ by hand shaking method and ether injection method respectively. In vitro release of Voriconazole from niosomes was very slow when compared to the release from pure Voriconazole solution. Drug release studies showed that the niosomal preparation was stable at refrigeration temperature (4^o C). The vesicles prepared by hand shaking method were found to be larger in size as compared to vesicles prepared by ether injection method. Almost constant drug release was observed in all formulations indicating zero order release pattern. Osmotic shock studies on vesicles showed that no significant change in the niosomal preparation was stored at normal saline.

From above these studies it was concluded that Voriconazole was successfully encapsulated into niosomes, Span 80 (1:1:3) vesicles prepared by hand shaking method showed best result in terms of encapsulation efficiency, in vitro drug release and to enhance the therapeutic effectiveness of Voriconazole, producing prolonged activity and simultaneously minimizing the side effects.

ACKNOWLEDGEMENT

The authors are thankful to mangement of Swami Vivekananda Institute of Pharmaceutical Sciences, Vangapally, Bongiri, Andhra Pradesh, India, for providing all the necessary facilities to carry out the work.

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