FORMULATION AND CHARACTERIZATION OF LONG CIRCULATING LIPOSOMES OF ANTI FUNGAL DRUG

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ABSTRACT

Voriconazole is an effective antifungal drug, used as first line treatment for Invasive Aspergillosis. The present work focusses on formulation of PEGylated liposomes to achieve longer circulation in the blood, prevent the liposomes from opsonisation by mononuclear phagocytic system of RES. The higher plasma concentration of voriconazole leads to visual disturbance and dermatological side effect, which are minimized by PEGylation. Compatibility studies like FTIR (Fourier Transform IR) and DSC (Differential Scanning Calorimetry) showed that lipids or drug do not interact and are highly compatible. Liposomes were prepared by thin lipid film hydration method, applying 3^2 full factorial design with various molar ratio of phospholipids like DPPC /Cholesterol /DSPE mPEG2000. Vesicular size and zeta potential was found within desired range of 100-300 nm to effectively pass through intra venous circulation. Long circulating liposomes were found to have entrapment efficiency of 75-85%. TEM (Transmission Electron Microscopy) showed distinctive spherical shaped vesicles. Long circulatory effect was confirmed from biodistribution study. Stability studies of long circulating liposomes were carried out and Pegylated liposomes successfully showed long circulatory action inside the body and minimized side effect of drug with appreciable antifungal activity determined by microbiological assay (zone of inhibition).

Keywords: Voriconazole, Invasive aspergillosis, Long Circulating Liposomes, Thin film hydration method.

INTRODUCTION

Introduction to disease

Aspergillosis [13] is a disease caused by Aspergillus species of fungus. The spectrum of illness includes allergic reactions, lung infections, and infections in other organs like kidney, brain etc.

Causes of Aspergillosis: [13]

Treatment with antibiotic drugs for a prolong period of time.

Individual with weakened immune systems (patients with HIV/AIDS)

Patients undergoing chemotherapy or steroids treatment (weakened immune system due to toxicity of these drugs)

Invasive aspergillosis

Patients with damaged or impaired immune systems die from the disease. Their chances of living are improved the earlier the diagnosis is made but unfortunately there is no good single diagnostic test. Often treatment has to be started as soon as the condition is suspected. This condition is usually clinically diagnosed in a person with low defences such as bone marrow transplant, low white cells after cancer treatment, AIDS, chronic granulomatous disease or major burns. People suffers with fever and symptoms affecting the lungs (cough, chest pain or breathlessness), which do not respond to standard antibiotics. X-rays and scans are usually abnormal and help to localise the disease. Bronchoscopy (inspection of the inside of the lung with a small tube inserted via the nose) is often used to help to confirm the diagnosis. Cultures and blood tests (especially antigen detection) are usually necessary to confirm the disease. In people with particularly poor immune systems, the fungus can transfer from the lung through the blood stream to the brain or to other organs, including the eye, the heart, the kidneys and the skin. Usually this is a bad sign as the condition is more severe and the person sicker with a higher risk of mortality. Pharmacological treatment includes antifungal drugs such as:

Voriconazole (first line treatment- oral/IV administration), caspofungin and micafungin (partially effective), itraconazole, (reports of resistance observed), amphotericin B (required in larger doses, also faced with major kidney and organ damage) and posaconazole (prophylactic).

Introduction to Long Circulating Liposomes [22, 23, 24, 25]

Conventional liposomes are faced with a limitation that they are removed from the circulation. A stealth liposome [23] is a spherical vesicle with membrane composed of phospholipids bilayer used to deliver drugs or genetic material into cells. Coating of liposomes with PEG reduces the rate of uptake by macrophages (stealth effects), avoid detection by immune system and leads to a prolonged presence of liposomes in the circulation and consequently provides ample time for the liposomes to escape from circulation through leaky endothelium.

Voriconazole is the drug of choice for invasive Aspergillosis in susceptible individuals. Severe dermatological (Steven-Johnson syndrome) side effect, phototoxicity and hepatotoxicity due to higher plasma concentration and longer duration of treatment has been reported. Hence, long circulating liposomes of voriconazole were formulated to increase the residence time, decrease dosing frequency and reduce opsonisation. Moreover aqueous stability of Voriconazole can be improved by liposomal formulation.

Materials and methods

Materials

Voriconazole was procured from Adventus Laboratories (India) Pvt. Ltd. (Baroda). Lipids: (DPPC) 1.2-Dipalmitoyl-sn-glycero-3-phosphocholine, (DSPE-MPEG-2000) 1,2Distearoyl-sn-glycero-3-phosphoethanolaminemethyl-polyethyleneglycolconjugate-2000 Na^+ salt was obtained from Lipoid GmbH, Ludwigshafen (Germany) and Cholesterol from Loba chemie Pvt. Ltd. (Mumbai) All the other chemicals were of analytical grade.

Formulation of Liposomes [54-59]

Vesicles were prepared by Film Hydration Method [21, 30, 62, 63], under reduced pressure. Different ratio of lipid and cholesterol were taken and Voriconazole was dissolved in a solvent mixture containing chloroform and methanol (9:1). The round bottom flask containing the solution was attached to the rotary evaporator (EQUITRON ROTA EVAPORATOR) under reduced pressure (500- 600 mm Hg), temperature and RPM as 50°C and 30 rpm respectively under inert environment of nitrogen. The flask is rotated till the solvent evaporated and thin, transparent film was formed on the walls of the flask. It was kept overnight under controlled vacuum and pressure. The film was then hydrated by 15 ml of phosphate buffer saline pH 7.4. The formed vesicles were sonicated for a specific time (as per design layout) in ice bath to avoid degradation. Sterilization of SUV was done by membrane filtration in aseptic condition. Vesicles were stored in vials. Stored SUV were lyophilized and reconstituted with Water for Injection.[64] **[Figure1]**

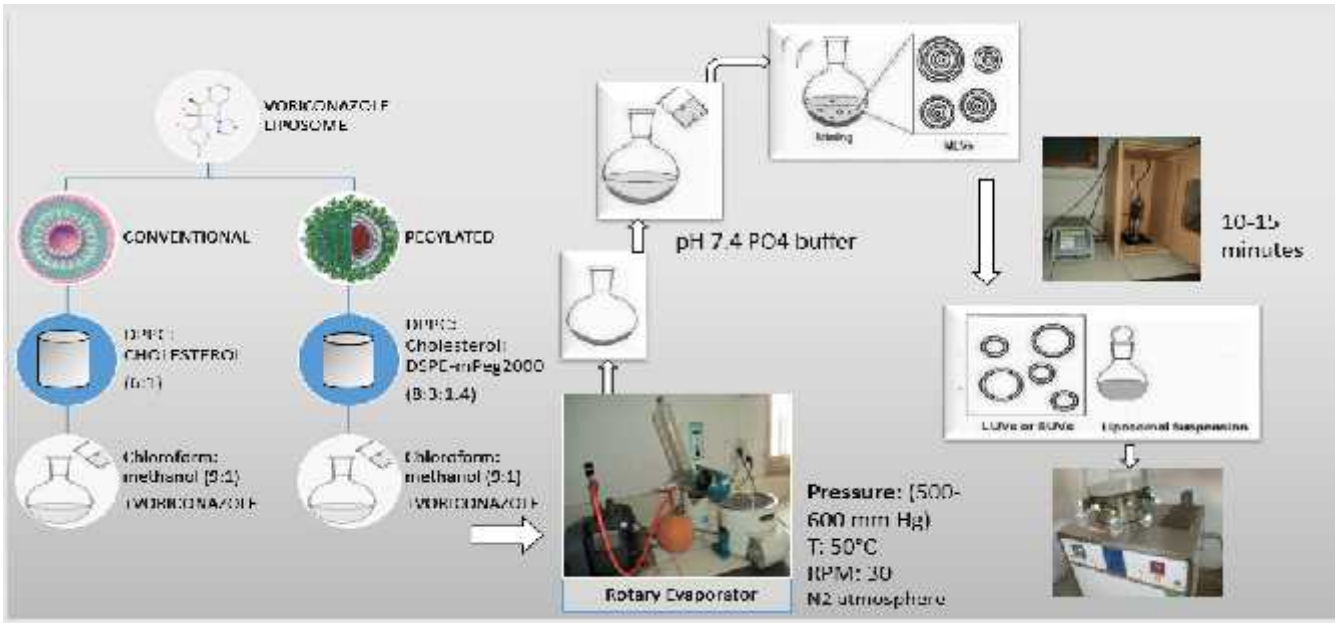

Figure 1: Formulation of Liposome of Voriconazole

Screening of processing parameters [60]

Screening of process parameter was done on basis of formulation process by trial and error method which affects on particle size and PDI of liposomes. Formulation parameters are following.

Speed of rotary evaporator (30, 50, 60, 90 rpm)
Temperature of rotary evaporator (50^0C, 60^0C)
Sonication cycles [pulses on/off (15/5), (30/30)].

OPTIMIZATION OF THE FORMULATION [54]

Experimental Design

In the research work 3^2 full factorial design was applied .The factors considered in experiment were, molar ratio of DPPC: Cholesterol and DPPC: Cholesterol: DSPE- mPEG 2000 another factor was sonication time and the responses measured were vesicle size and % entrapment efficiency. The 3 levels for 2 factor for Long Circulating Liposomes were coded as -1, 0, +1 respectively. Conventional Liposomes were also prepared with thin film hydration method and that would be used for comparison of in vitro release study of liposomes.

For Conventional Liposomes: three batches with **molar ratio of DPPC: Cholesterol** was **6:1, 7:2, 8:3** and the corresponding **sonication time was 5, 10 and 15 minutes** respectively.

For Long Circulating Liposomes:
Levels in Coded Value: -1, 0, +1.
Factor A (Molar ratio DPPC: Cholesterol: DSPE-mPEG 2000): (6:1:0.2), (7:2:0.8), (8:3:1.4)

Factor B (sonication time): 5, 10, 15 minutes respectively.

The above factors with their levels and response of each of the experiments were fed in the design expert software 7.0 and the further analysis of the data was done using the software.

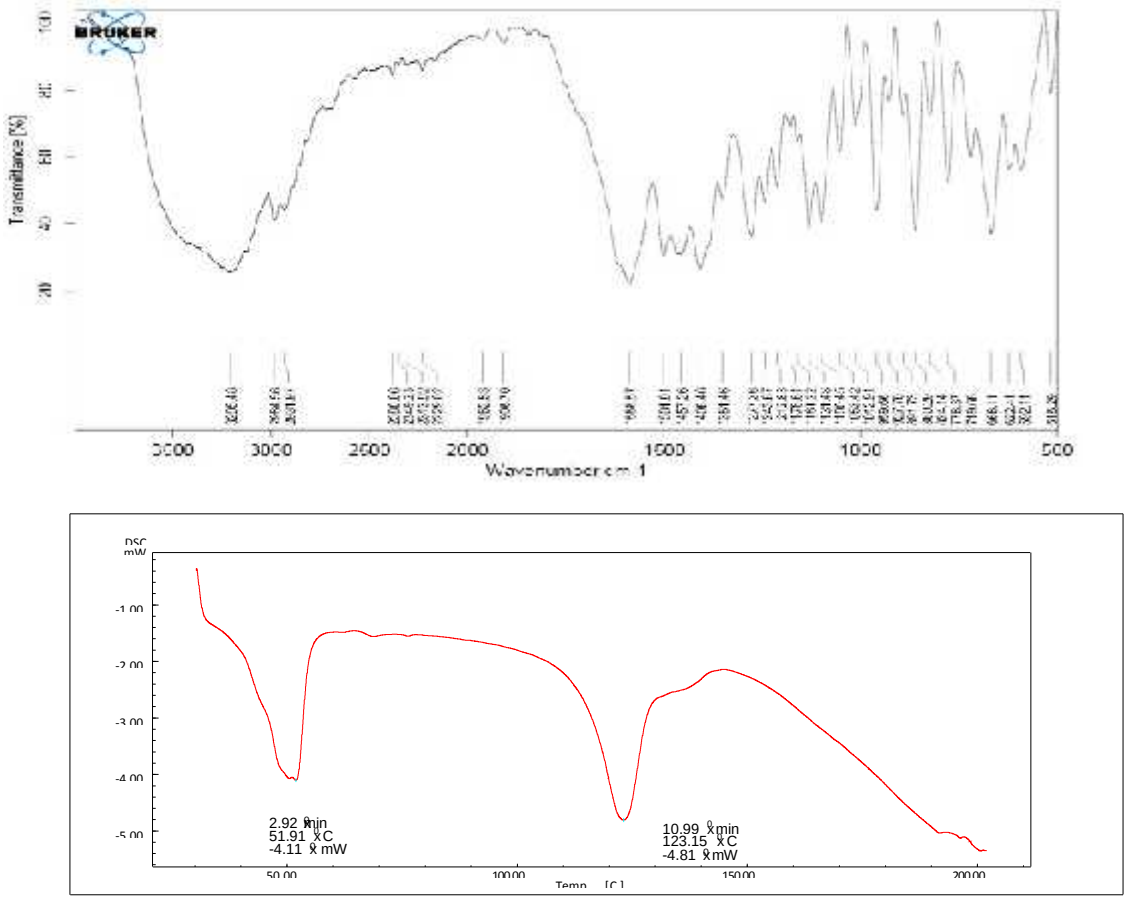

Figure 2: Drug Excipient study (FTIR spectra and DSC analysis of drug-lipid mixture)

METHOD OF ESTIMATION OF VORICONAZOLE

UV-Visible Spectroscopy method

Voriconazole was estimated by UV-Visible spectrophotometry using phosphate buffer saline (PBS) pH 7.4. To determine absorption maxima 10µg/ml solution was prepared and scanned between 200-4000 nm using spectrophotometer (Shimadzu 1800, UV-Visible double beam Spectrophotometer). For preparation of calibration curve, the aliquots of 0.5, 1, 1.5, 2, 2.5, 3, 3.5 ml of the stock (0.1 mg/ml) were serially diluted with 10 ml of PBS to get 5, 10, 15, 20, 25, 30 µg/ml concentration. Absorbance of each solution was measured at 255 nm against PBS as a blank. The data were subjected to linear regression analysis.

HPLC method for estimation of Voriconazole [53]

A rapid and sensitive RP–HPLC method with UV detection (256 nm) for routine analysis of voriconazole was developed. Chromatography was performed with mobile phase acetonitrile: water (50:50 v/v) with flow rate of 1.0 ml min^{-1}. The calibration curve was plotted with five concentrations 5–25 μg/ml solutions of voriconazole, prepared in acetonitrile: water (50:50 v/v) mixture.

Drug-Excipient Compatibility study

FTIR study for drug-Lipid interaction [51]

The samples of pure drug Voriconazole, DPPC, DSPE-mPEG2000 and Cholesterol individually and drug-lipid combination (mixed with IR grade KBr) were prepared. All the samples were placed between IR transmitting windows in FTIR Spectrophotometer (Bruker, ALPHA- T) and scanned over a range of 4000 to 400 cm^{-1}.

DSC study for drug-Lipid interaction [50, 51, 52]

The DSC study was performed of pure drug, DSPE-mPEG2000 and mixture (Drug: Cholesterol: DPPC: DSPE-mPEG2000 in ratio of 1:1:1:1). Samples were taken in standard aluminium pan and heated for 30 minutes at a rate of 10°C/min till 300°C under constant nitrogen stream (20 ml/min).

EVALUATION OF FORMULATION

Transmission Electron Microscopy

Negative electron microscopy (Tecnai 20, Philips, Holland) technique was used to study liposomal shape and lamellarity.

Vesicle Size and Size Distribution [66, 68]

Size and size distribution of vesicles was determined by photon correlation spectroscopy (PCS) using Zetasizer Nanoseries (Malvern Instruments, Malvern and Worcestershire, UK). Each sample was taken in the disposable cuvette and analysis was performed at 25^0C with an angle of detection of 90^0.

Table 1: Result obtained for long circuating liposomes

Run	Factor A	Factor B	Vesicle size (nm)	Zeta potential (mV)	PDI	%Entrapment efficiency
1	-1	-1	311.1±1.3	-7.2	0.507±0.003	58.05±0.51
2	0	-1	274.9±1.2	-8	0.622±0.004	69.45±0.50
3	1	-1	198.0±0.9	-11.6	0.496±0.007	79.05±0.47
4	-1	0	178.2±1.2	-8.0	0.478±0.005	62.85±0.23
5	0	0	167.9±1.1	-10.3	0.485±0.007	69.15±0.33
6	1	0	154.8±1.3	-15.0	0.443±0.003	82.05±0.47
7	-1	1	160.8±1.4	-11.3	0.449±0.002	67.05±0.76
8	0	1	143.6±1.1	-16.4	0.396±0.004	72.6±0.69
9	1	1	129.6±1.2	-17.3	0.366±0.006	84.45±0.41

Zeta-Potential [66, 67]

Zeta potential (ZP) of vesicular dispersion was determined using Zetasizer Nanoseries (Malvern Instruments, Malvern, Worcestershire, UK). Charge on vesicles and their mean ZP values with standard deviation (±S.D.) were obtained directly from the instrument. Particles having high zeta potential value show higher stability, as the particles of the same formulation having same charge on it will repel each other, and will avoid aggregation.

Entrapment Efficiency [66, 68, 69]

The extruded liposomal samples were centrifuged at 3,000 rpm in refrigerated centrifuge at 4°C to pelletize the unencapsulated drug. The supernatant was centrifuged at 10,000 rpm to pelletize the drug loaded liposomes. Absolute alcohol (1ml) was used to disrupt the liposomes. The pellets were resuspended and diluted with PBS pH 7.4. The concentration of the encapsulated drug was measured as absorbance at 255 nm using UV-visible spectrophotometer (Shimadzu 1800, UV-Visible double beam Spectrophotometer). The absorbance was converted into drug concentration using a standard curve. The encapsulation efficiency was calculated as:

$$\%\ Encapsulation\ Efficiency = \frac{Encapsulated\ drug}{Total\ drug} * 100$$

In Vitro release Study [56, 68, 70]

Diffusion Medium: pH 7.4 phosphate buffer saline
Membrane for permeation: Dialysis membrane 50
Temperature: 37°C
Sampling Interval: 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, 20, 22 and 24 hrs
Volume of Diffusion Medium: 150 ml

In- Vitro drug release study was performed in Dialysis membrane 50 with solution in it and tied at both the end with a thread. The dialysis bag was put into a beaker containing 150 ml of phosphate buffer saline pH 7.4. The whole system was kept at 37°C and stirred continuously by magnetic stirrer. At a sampling interval time 5 ml of the aliquot was withdrawn and the absorbance of the aliquot was measured in UV Spectrophotometer. 5 ml of the Phosphate buffer saline 7.4 was replaced to the system to maintain sink condition.

Antifungal activity [71-74]

The antimicrobial studies of the optimized formulations and of the marketed formulation against *Aspergillus niger* were performed. For the development of inoculum, Lyophilized strip of strain of *A. niger* (ATCC 16888) was subcultured in nutrient broth and incubated at 25 ^{0}C for 48 hrs. Potato dextrose agar media seeded with the strain of *A. niger* (ATCC 16888) was allowed to solidify in the sterilized petri plate. Voriconazole drug solution was

placed into the bore created in potato dextrose agar at suitable distance and plates were incubated at 25 ^{0}C for 48 hrs.The diameters of the zones of inhibition were measured.

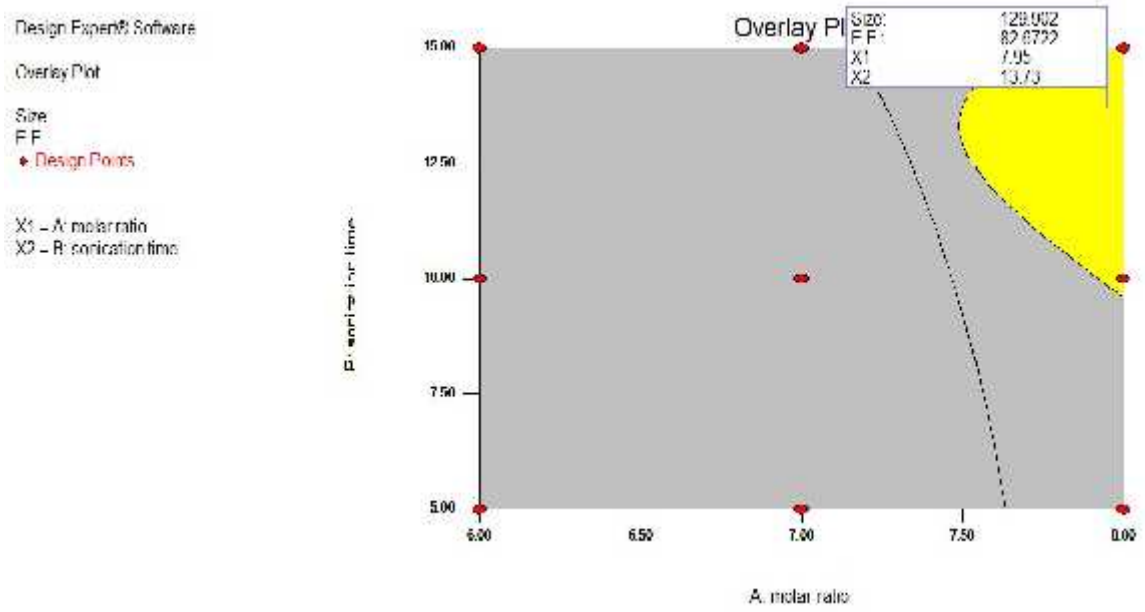

Figure 3: Optimization of formulation (3D plots for vesicle size & % EE and overlay plot for checkpoint batch preparation)

Biodistribution study

Bioanalytical Assay of Voriconazole in rat plasma [75, 76]

Bio analytical assay of Voriconazole in rat plasma was carried out by RP-HPLC method. The calibration curve of samples were prepared by spiking 100μL of Wistar rat plasma and mixing with various concentration of the appropriate standard working solution prepared to obtain VRC final concentrations of 50, 100, 250, 500, 1000 and 2500 ng/ml. Standard stock solution of Ketoconazole (Internal Standard) was prepared at 500μg/mL similarly. This solution was successively diluted to result in a final concentration of 4μg/mL of Ketoconazole. Prior to the chromatographic analysis, all samples of Voriconazole and IS were deproteinized by addition of ice-cold methanol and vortexed for 30 sec and centrifuged at 12,000 rpm, 4 ◦C for 15 min. Supernant solution was collected and diluted with proper mobile phase and chromatographic analysis was carried out.

In vivo animal study [56, 77, 78]

Animal experiments were carried out according to IAEC (Institutional Animal Ethical Committee) containing Protocol No: *PIPH 7/14*. The rats were housed under standard conditions and had *ad libitum* access to water and standard laboratory diet. Voriconazole solution of marketed preparation and optimized long circulating liposomal formulation of voriconazole was administered intravenously by tail vein in Wistar rats. Blood sample was collected from retro orbital plexus of lightly anaesthetised rats at 1hr, 6hr, and 24 hr. Animals were euthanized and tissues (lungs and kidney) were isolated and homogenized by homogenizer (Silent Crusher M-Heidolph). The supernatant (plasma) was taken and mixed with methanol and centrifuged in refrigerated centrifuge to isolate plasma. The resulting supernatant was subjected to HPLC (LC-10ADVP - Shimadzu) for analysis.

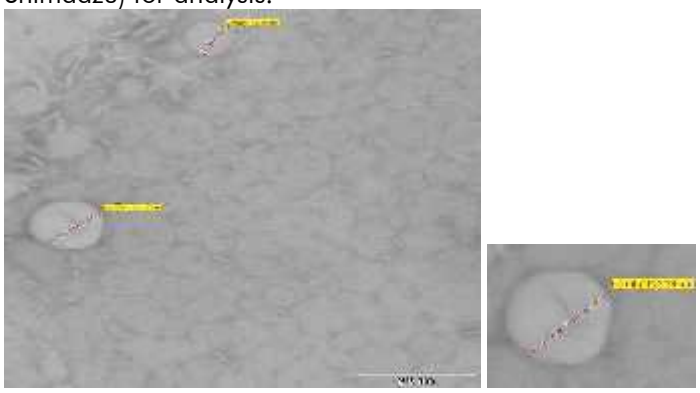

Figure 4: Transmission Electron Microscopy of Optimized batch. [56]

Stability studies [68, 79]

Stability study of long circulating liposomes was done at refrigerated storage condition 5 ± 2^{0}C and 25 ± 2^{0}C at 65% RH ± 5% RH under accelerated stability study in Humidity controlled oven (Remi Instruments Ltd). The particle size, size distribution and drug encapsulation efficiency were measured periodically.

Result and discussion

Method of estimation of Voriconazole (UV and HPLC method)

UV Spectroscopy of drug Voriconazole in Phosphate Buffer Saline in pH 7.4 λ_{max} of Voriconazole was found at 255 nm. This (λ values) was selected for rest of the analysis. From the calibration curve, regression analysis gave equation as y =0.025x-0.035 (R^2=0.998).

A calibration curve obtained from HPLC method followed by regression analysis gave
$y = 15.99x + 27.07$ ($R^2 = 0.995$)

Drug-Excipient Compatibility study:

FTIR study for drug-Lipid interaction: [80] [Figure 2]
The spectra of individual components as well as the combination of drug lipid were obtained. From the spectra the following peaks were observed:

Alcohol (-OH) **3415.15 cm^{-1}**
Alkane (-CH_3) **2985.86 cm^{-1}**
Imines (-C=N-) **1586.07 cm^{-1}**
Fluoride (Ar-F) **1013.59 cm^{-1}**
Ester (C=O) **1735.64 cm^{-1}**
Phosphate ester (P=O) **1163.08 cm^{-1}**

From the spectra, peak of drug is distinctly obtained in the physical mixture. There is no interaction between the drug and lipids indicating drugs and lipids are compatible

DSC study

DSC thermogram of physical mixture showed distinct endothermic peak obtained at 51.91^{0}C (of DSPEmPEG-2000) and 123.15^{0}C (of Voriconazole). From the DSC thermogram, no unknown endothermic peak obtained in the thermograph of the physical mixture of pure drug and lipids. Thermogram of overlay spectra of DSC showed two distinct endothermic peak. So, DSC studies confirm that there is no physical or chemical interaction between drugs and lipids.

Screening of process parameters:

Screening was done on the basis of formulation parameters which affect size and PDI of liposomal formulation. From screening study, it was found that as RPM of rotary evaporator was increased, the size of liposomal formulation increases and uniform film was not formed at the round bottomed glass surface, PDI was increased with size and %E.E. was decrease. So, less RPM of rotary evaporator give uniform film and best result for size and %E.E. Degradation of lipids with increase in temperature was observed. Sonication cycles mean Pulse on/off of probe sonicator that is 15/5 sec (15 sec. on and 5 sec. off) gave best result of size and PDI and %E.E. So, these parameters with best result were used for further studies in formulation of conventional and Pegylated liposomes.

Conventional Liposomes with Vesicle size, Zeta potential, PDI and % Entrapment Efficiency.

Molar ratio of DPPC: Cholesterol (6:1, 7:2, 8:3)
Sonication time (min) (5, 10, 15). Molar ratio and sonication time affects vesicle size and %E.E. As with very low sonication time, vesicle size was increased and % entrapment efficiency is less. And with high sonication time, vesicle size was decreased and % Entrapment efficiency is more.

Long Circulating Liposomes with Vesicle size, Zeta potential, PDI and % Entrapment Efficiency.

Factor A: Molar ratio of DPPC: DSPE-mPEG 2000: Cholesterol (6:1:0.2, 7:2:0.8, 8:3:1)
Factor B: Sonication time (min) (5, 10, and 15)

Table: Result obtained for Long Circulating Liposomes

Optimization of long circulating liposomes:

Optimization of long circulating liposomes was done by 3^2 full factorial designs. For long circulating liposomes two factors were molar ratio of DPPC: CHOL: DSPE-mPEG-2000 and Sonication time. The responses measured were Vesicle size and % E.E. after reconstitution of lyophilized product with water for injection. ANOVA was applied and the following results were obtained. [Table 1]

Effect on Vesicle size:

ANOVA for Vesicle size

$Size = + 171.44 - 27.95 * A - 58.33 * B + 20.48 * A * B - 6.72 * A^2 + 36.03 * B^2$ **(Full model ANOVA)**

$Size = 190.98 - 34.55 * A - 55.033 * B$ **(Reduced model ANOVA)**

From ANOVA, p value is < 0.05, the full and reduced model are significant. The final equation shows the effects of different factors on the size. Sonication time has the maximum effect on vesicle size is the highest as among the all β values. As the value is negative the effect i.e. as we will increase the sonication time, the vesicle size will be decreased. Effect of molar ratio is also positive on the vesicle size. As the β value is negative the effect i.e. as we will increase the molar ratio the vesicle size will be decreased.

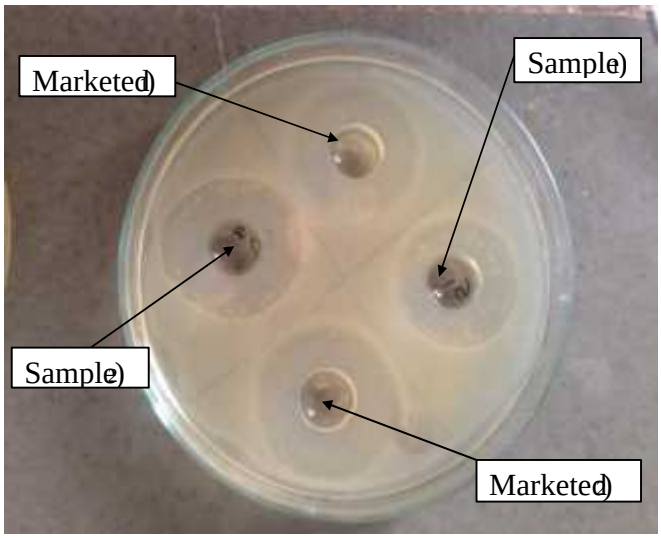

Figure 5: Zone of Inhibition of Voriconazole

% Entrapment Efficiency:

ANOVA for % E.E

$\%E.E. = + 70.12 + 9.6 * A + 2.93 * B - 0.90 * A * B + 1.85 * A^2 + 0.42 * B^2$ **(Full model ANOVA)**

$\%E.E. = 71.394 + 8.466 * A + 1.608 * B$ **(Reduced model ANOVA)**

The full and reduced model are significant. The final equation shows the effects of different factors on the %E.E. The β value is positive the effect i.e. as we will increase the molar ratio the vesicle size will be

increased. The β value of sonication time is positive effect on %E.E. i.e. as we will increase the sonication time, the vesicle size will be increased. Effect of molar ratio is also positive on %E.E.

PREPARATION OF THE CHECK POINT BATCH [59]

Using the design expert, the design space was prepared which gave size range from 120 - 140 nm and % E.E. from 75 - 85% and from the resulting design space, randomly one flag point was selected and the batch was prepared and evaluated for size and % E.E. The resulting parameters were compared with the standards provided by the software. When we had narrowed down the size selection region from 125-140 nm and % E. E range selected was 75-85% the design expert has shown the following design space that will give the desired range of result.

Results of check point batch: By selecting any point from the design space a batch was prepared. The vesicle size and % EE was found to be 126.7±0.8 nm (predicted: 129.902 nm) and 80.55±0.88% (82.96 %) respectively.

Table 2: Invitro drug release comparison

Time (hr)	% cumulative drug release study of formulation		
	Marketed preparation	Conventional liposome	Long Circulating Liposomes
0.5	54.188 ± 0.50	23.063 ± 0.45	13.875 ± 0.540
1	70.394 ±0.90	38.938 ± 0.84	28.169 ± 0.780
2	80.488 ± 1.20	52.188 ± 0.98	35.963 ± 0.920
3	86.419 ± 1.30	61.125 ± 1.08	41.819 ± 1.100
4	88.700 ± 1.40	68.375 ± 1.30	47.619 ± 1.250
5	89.706 ± 1.55	72.669 ± 1.39	55.744 ± 1.390
6	91.319 ± 1.68	75.319 ± 1.48	61.813 ± 1.467
8	92.95 ± 1.87	81.794 ± 1.62	69.944 ± 1.602
12		87.356 ± 1.79	75.238 ± 1.652
16			80.631 ± 1.712
20			84.481 ± 1.759
22			86.219 ± 1.779
24			89.344 ± 2.000

Transmission Electron Microscopy [Figure 4]

The average vesicular size and zeta potential of the optimized batch obtained from Zeta sizer instrument was 126.7±0.8 nm and -17.3 respectively.

Invitro Release Study

Release study of the liposomal formulation and marketed formulation was performed using dialysis membrane, the following table shows the % CDR with time. In case of free Voriconazole more than 92.95 % drug was released within 8 hr. and Conventional liposomal formulations produced slower voriconazole release was more than 87.365 % within 12 hr. whereas long circulating liposomal formulations produced slower voriconazole release was more than 89.344 % within 24 hr. [Table 2]

Antifungal activity

Marketed Formulation showed average diameter of 18.3 mm and liposomal formulation showed average diameter of 18.4 mm inhibition which is similar to the zone of inhibition of marketed formulation of liposomal formulation. [Figure 5]

Biodistribution study

After 1hr, 6hr and 24hr, drug was extracted out from blood plasma and analysed using HPLC. In case of long circulating liposomal injection plasma peaks obtained at 1hr, 6hr and 24 hr with increase in peak area. Hence, it is concluded that the pegylated liposomes exhibits long circulation in the body. This was also confirmed by peak area of drug in HPLC chromatogram.

Time (hr)	Peak Area (mV.s)	
	Marketed Formulation	Pegylated Liposomes
1	227028	102935
6	114131	185672
24	2339	215689

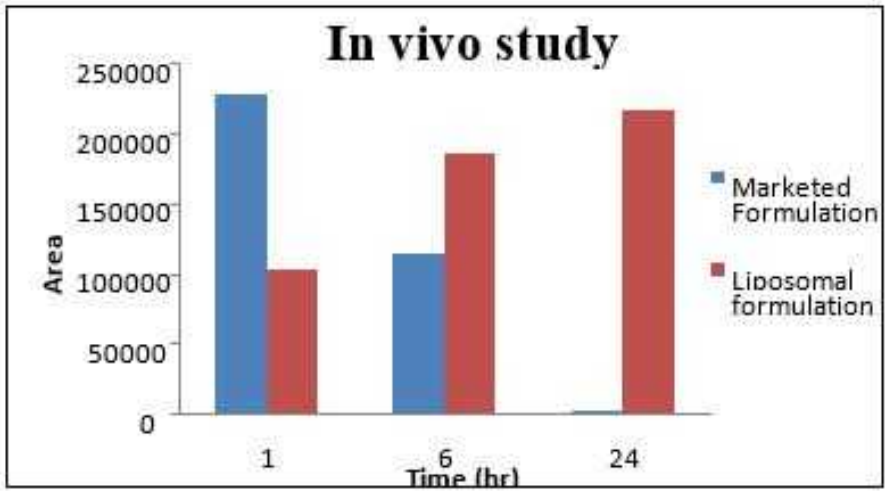

Figure 6: Comparison of Marketed formulation and Liposomal formulation

Stability study

Table 3: Stability study data at 5° ± 2° C; 60% ± 5% RH

Duration	Storage Condition	Vesicle Size (nm)	% Entrapment Efficiency
0th day	5 ± 2° C; 60% ± 5% RH	127.23±1.5	80.55±0.28
15th day	5 ± 2° C; 60% ± 5% RH	132.5±1.8	78.85± 0.42
30th day	5 ± 2° C; 60% ± 5% RH	148.8±2.3	72.65± 0.39

Stability study was carried for a month for long circulating Liposomes. Formulation was stable at temperature of 5 ± 2° C and Relative Humidity of 60% ± 5%. There was no significant change in the Entrapment Efficiency of the drug or the size of the liposomes. However, when formulation of liposomes were subjected to 25° ± 2° C and 60% ± 5%. There was loss of liposomal structure and entrapment efficiency.

Conclusion

The objective of present work was to prepare and evaluate long circulating liposomes of antifungal drug. Long Circulating Liposomes containing Voriconazole were prepared by Thin Film Hydration technique. In present study, 3^2 full factorial design was applied in which molar ratio and sonication time were taken as independent variables and Vesicle size and % Entrapment Efficiency as dependent variables. The optimized formulation showed the particle size around 126.7±0.8 nm with entrapment efficiency 80.55±0.88 %. In-vitro release study shows that liposomes seem to be a sustained dosage form of Voriconazole vital for treatment of Invasive Aspergillosis. Zone of Inhibition optimized formulation showed that voriconazole exhibited anti fungal activity which was found almost similar to marketed formulation. Long circulatory effect was successfully found out from biodistribution study. Optimized formulation exhibited good stability during 1 month storage at 5° C ± 2° C at 65% ± 5% RH. Thus, it was concluded that Voriconazole loaded long circulating liposomes were suitable and desired dosage form for the treatment of Invasive Aspergillosis.

Acknowledgement

We are very thankful to Parul University for providing infrastructure for conducting research work

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