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RESEARCH ARTICLE

HP-β-CD BASED VORICONAZOLE NIOSOME FOR OCULAR DRUG DELIVERY: IN VITRO, ANTIFUNGAL & STABILITY ASSESSMENT

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ABSTRACT

The main objective of drug delivery system to the eye is to improve existing ocular dosage forms and exploit newer drug delivery system for improving the therapeutic efficiency. The present study was to prepare & evaluate Voriconazole Niosome using non ionic surfactant like Span 60, Tween 80 & Brijj 30 in the presence of cholesterol & a negative charge inducer dicetyl phosphate (DCP) in different molar ratios & by employing a thin film hydration technique & were investigated morphology, entrapment efficacy, zeta potential, particle size, in-vitro release, physical stability, antifungal activity The entrapment efficiency (EE %) & in-vitro drug release of all formulation follows decreasing order: Span 60>Tween80> Brijj 30.The formulation VN-2 (1:1:1) molar ratio of Span 60, cholesterol & DCP gave the most advantageous entrapment (84.08±1.00 %) & release rate(Q_{8h} = 84.34±2.09%) as compared to other compositions. The study concluded that the Voriconazole loaded niosomes to be effective in sustaining the drug release leading to decreased side effects & increased patient compliance.

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INTRODUCTION

The main objective of drug delivery system to the eye is to improve existing ocular dosage forms and exploit newer drug delivery system for improving the therapeutic efficiency. Topical application of eye drops is the most common method of administering drugs to the eye in the treatment of ocular diseases. Topical and localized applications are still an acceptable and preferred route, such dosage forms are no longer sufficient to overcome the various ocular diseases like glaucoma due to poor bioavailability, due to the efficient mechanism protecting the eye from harmful materials and agents. (Saettone and Salminen, 1995; Arora *et al*., 2012; Rathore, 2010) This includes reflex, blinking, lachrymation, tear turnover, and drainage of tear results in the rapid removal of the drug from eye surface. Similarly frequent instillation of concentrated medication is required at the site of action which is patient incompliance. Vesicular drug delivery systems allows the entrapment of drug molecule into lipid bilayer or surfactant vesicles and thus increase drug concentration at the site of application with sustained drug delivery of medicament, which results in improved bioavailability. Such vesicles (liposome and niosome) acts as carrier for controlled ocular drug delivery by preventing metabolism of drug from enzymes present at the corneal epithelial surface. (Gaudana *et al*., 2009; Gannu and Rajeshwarrao, 2011; Modi and Shelat, 2012) Niosomes can encapsulate both lipophilic and hydrophilic drugs and protect against acidic and enzymatic effects in vivo. They offer several advantages over liposomes such as higher chemical stability, intrinsic skin penetration enhancing properties and lower costs. However, there may be problems of physical instability of niosomes during the storage, which includes vesicles aggregation, fusion, leaking or hydrolysis of encapsulated drugs. This may affect the shelf life of the niosomes. The niosomal drug delivery system have great advantage for poorly soluble drug by increasing its solubility, controlling its release and prolong its activity over period of time, Hence decreasing the frequency of administration and improving patient compliance . (Bruntn *et al*., 2009; Kaur *et al*., 2000; Kaur *et al*., 2008)

The fundamental requirements for the success of successful niosomes used in ocular delivery may be summarized as follows:-

Physician acceptance, User acceptance, Ease of handling and application, Patient comfort, lack of expulsion during application, lack of toxicity, noninterference with vision and oxygen permeability, reproducibility of release kinetics, applicability to a variety of drugs, sterility, stability, ease of manufacture, reasonable price and duration of action. (Budai *et al*., 2007; Robinson and Mitra, 1993; Azeem *et al*., 2009)

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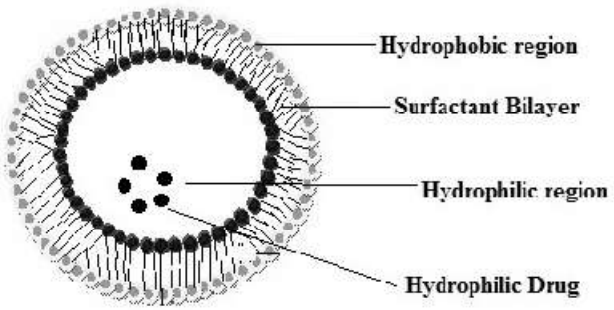

Fig.1. Diagrammatic view of Niosomal vesicular carrier

The novelty of research work is to prepare newest triazole derivative loaded niosome for the effective management of invasive aspergillosis and other fungal infection and achieves a sustained release profile suitable for ocular delivery with enhanced efficacy, which could overcome the drawbacks of conventional drug delivery. (Ranjan *et al*., 2014; Baillie *et al*., 1985; Lang *et al*., 2002)

Experimental section

Voriconazole was a kind gift sample from Glenmark Pvt. Ltd (Mumbai, India). Cholesterol was supplied by Fisher Scientific (Mumbai, India). The non-ionic surfactants viz. Span 40, Span 60, Span 80, Tween 60, Tween 80 and Brij 30, Brijj 72 were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India) and all the ingredients used in the procedures were of analytical grade.

Method of Preparation of voriconazole niosomes

The nano sized vesicles were prepared by using thin film hydration method. The composition of different formulation shown in table no.1 The specified quantities of cholesterol, non-ionic surfactant and dicetyl phosphate (DCP) were completely dissolved in 10 ml chloroform contained in a clean and dry round bottom flask (Table no.1). The transparent solution was reduced to a thin dry film using rotary evaporator (Perfit, India) at 50.00±2.00°C. Voriconazole was dissolved in phosphate buffer pH 6.8 with presence of HP-β-CD and the thin dry film was hydrated using this buffered drug solution. The film is allowed to hydrate for about 1 hour for the formation of niosomes. Milky dispersion is prepared which is kept at 4°C for 24 hours for maturation of the formed vesicles. (Abdelbary and El-Gendy, 2008)

Determination of entrapment efficiency of Voricanazole

The entrapment efficiency of the matured niosomes was determined using centrifugation method. The measured volume 5 ml of the prepared dispersion was centrifuged using cooling centrifuge (RIS-24BL, REMI, India) at 6°C for 1 hr to separate the free drug from niosomes. The niosomes formed a cake floating at the top of tube and clear solvent containing the unentrapped drug remained at the bottom. The cake was resuspended in 5 ml phosphate buffer, pH 6.8 and the process was repeated twice by centrifugation for 30 minutes to ensure complete removal of free drug. After suitable dilution with phosphate buffer, pH 6.8 the clear fraction was used for the determination of free drug spectrophotometrically by UV/visible spectrophotometer (Lab, India) (Kaur *et al*., 2004). The entrapment efficiency was calculated using the formula. (Perrie *et al*., 2004)

$$\% \text{ drug entrapment} = \frac{\text{Total drug} - \text{drug in supernatant}}{\text{Total drug}} \times 100$$

Zeta potential measurement & particle size measurement

Zeta potential of suitably diluted niosome dispersion was determined using zeta potential analyzer based on electrophoretic light scattering and laser doppler velocimetry method using zetasizer (Malvern instruments ltd. Worcestershire, UK) The temperature was set at 25 °C. Charge on vesicles and their mean zeta potential values with standard deviation of 5 measurements were obtained directly from the measurement. The z-average diameter of sonicated vesicles was determined by dynamic light scattering using a zetasizer nano ZS-90(Malvern instruments ltd., UK) for the measurement, 100 µl of the formulation was diluted with an appropriate volume of phosphate buffer pH 6.8 and the vesicle diameter and polydispersity index were determined. (Zhang *et al*., 2007)

In *vitro* drug release study

The *in-vitro* drug release pattern was studied using modified USP dissolution apparatus. Samples were placed on the dialysis membrane previously soaked over-night in phosphate buffer, pH 6.8 and attached to lower end of a glass tube. The tubes were immersed in dissolution vessel containing phosphate buffer, pH 6.8 maintained at 37±0.5°C. Samples were withdrawn at regular interval of time and replaced with equal amount of phosphate buffer pH 6.8 to maintain sink condition. The samples were analyzed by UV/visible spectrophotometer (Lab, India) for the drug release pattern. The study was continued up to 8 hours. (Ruckmani and Sankar, 2010)

Stability studies

Physical stability of the niosomes was studied by leaching of the drug from the vesicles in the native prepared form i.e. dispersion stored under refrigeration. The optimized dispersion with the composition of cholesterol and span in 100:100 molar ratio with DCP was sealed in glass vials and stored under refrigeration temperature (2-8°C) for a period of 90 days. Samples were withdrawn at definite intervals of time and the amount of drug remaining was calculated by the method employed for entrapment efficiency (%) determination (Yadav and Ahuja, 2010; Motwani *et al*., 2008).

Antifungal Studies

Sabouraud dextrose agar (mycological peptone 10mg, dextrose 10mg) was used to prepare the medium. Sabouraud dextrose agar was used in quantity of 65mg and dissolved by heating in one liter of distilled water with frequent agitation and boiled for 1min to completely dissolve the powder. Autoclave the media at 121°C for 15min. The media were poured into 200 mm diameter plastic plates and left to solidify for 15min. Wells of 10mm diameter were punched out using a steel borer. The antifungal activity of voriconazole loded noisome with or without dicetyl phosphate (VN2/VN4), marketed preparation (Vozole1%w/v) was measured by plate microbioassay (agar cup diffusion) method. (Pawar *et al*., 2013)

RESULTS AND DISCUSSION

The niosome vesicles were prepared by span 60, Tween 80, span 80, Brijj 30 and optimized on the basis of temperature control and ratio of different excipients. The niosomes were not formed at room temperature because 55-60^{0}C temperature is needed for complete dissolution of cholesterol, and at temperature 70-85^{0}C vesicles not formed because vesicles were broken when seen in light microscope. Niosomes were formed at 55°C-60°C. (Agarwal *et al*., 2001; Kaur *et al*., 2004) Result shown in Table 1.

centrifugation, the entrapment efficiency of all formulations was studied. From this study, it was found that the amount to drug entrapped in niosomes ranged between 84.08±1.00% for VN-2 formulation to 76.64 ±1.14% for VN-3 formulation. Hence, the niosome formulated with span 60 were found to be optimum for loading maximum amount of voricanazole in niosomal formulation. (Baillie *et al*., 1985; Ruckmani and Sankar, 2010; Pardakhti *et al*., 2007) Result shown in Table 2. The release pattern of Voriconazole form prepared niosome have been affected presense & absence of charge inducer component that is dicetyl phosphate. Voriconazol based niosome containing span 60 with optimize ratio of lipid

Table 1. Formulations of niosome containing Voricanazole

Formulation code	Cholesterol (%mol/mol)	Surfactant (mol/mol)	Dicetyl phosphate (%)
VN1	0.5	Span 60 (1)	0.5
VN2	1	Span 60 (1)	1
VN3	0.5	Span 60 (1)	-
VN4	1	Span 60 (1)	-
VN5	0.5	Tween 80 (1)	0.5
VN6	1	Tween 80 (1)	1
VN7	0.5	Tween 80 (1)	-
VN8	1	Tween 80 (1)	-
VN9	0.5	Brijj 30 (1)	0.5
VN10	1	Brijj 30 (1)	1
VN11	0.5	Brijj 30 (1)	-
VN12	1	Brijj 30 (1)	-

Table 2. Characterization of niosomal formulations

S.No.	Batches	Entrapment Efficiency (%)	Zeta potential	Particle Size (nm)
1.	VN-1	80.26	-29.71	634.33
2.	VN-2	84.08	-30.10	647.21
3.	VN-3	76.64	-29.63	665.57
4.	VN-4	72.25	-28.93	574.65
5.	VN-5	60.91	-29.86	403.17
6.	VN-6	68.13	-26.46	438.67
7.	VN-7	62.23	-28.96	429.13
8.	VN-8	58.13	-25.83	425.89
9.	VN-9	54.50	-23.46	335.91
10.	VN-10	60.17	-21.6	343.74
11.	VN-11	52.23	-15.46	322.16
12.	VN-12	55.13	-18.6	313.74

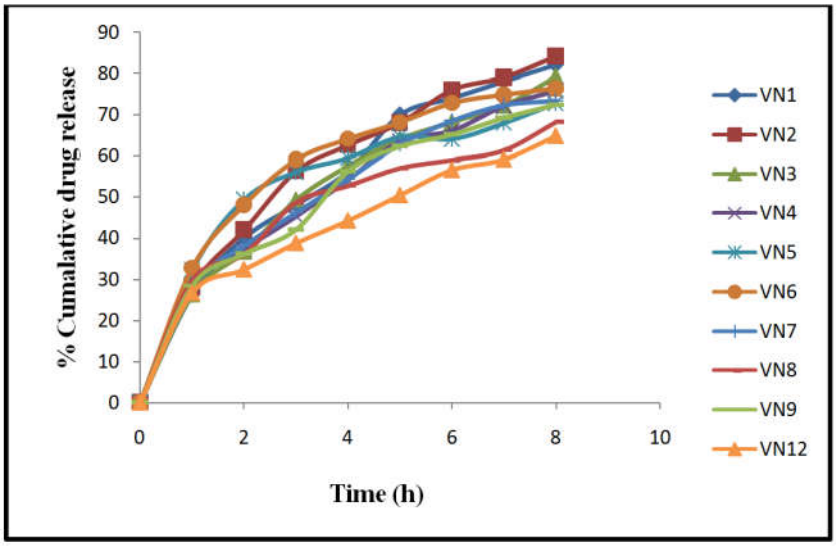

Fig.2. Graph of %CDR v/s Time of formulations VN-1 to VN-12

Niosomes were subjected for photomicroscopic study for characterizing size distribution of niosomes from this study it was found that the average particle size was 647.21 ± 3.08 nm for VN-2 formulation. After removal of unentrapped drug by

component with DCP (VN2) provide maximum drug release as compare to other formulations. These could be due to contribution of an alkyl chain length and HLB value of surfactant present in formulation. Span 60 containing lower

HLB value & bulky saturated alkyl side chain length affect significantly on drug release rate results in higher percentage of drug release from niosome.

could be contribute sustain release (Maiti *et al.*). The formulation investigated for physical stability studies showed the residual drug content in the niosomes to be 77.61% at the

Table 3. Drug release profile of all formulations

Time (h)	% Cumulative Release											
	VN1	VN2	VN3	VN4	VN5	VN6	VN7	VN8	VN9	VN10	VN11	VN12
1	26.33	28.21	26.43	28.74	32.32	32.86	28.25	29.82	28.43	27.21	25.43	26.74
2	40.15	42.12	36.82	37.46	49.54	48.25	38.24	36.26	36.2	40.41	29.45	32.45
3	48.27	56.41	49.45	45.45	56.22	59.19	46.29	48.86	42.18	48.52	44.28	38.86
4	56.36	62.84	57.72	54.35	59.62	64.22	54.42	52.89	56.36	52.84	57.72	44.35
5	70.18	68.25	64.32	63.65	64.72	68.21	62.82	57.16	62.29	59.17	62.52	50.54
6	74.22	76.24	68.43	66.18	64.18	72.88	68.46	59.12	65.13	63.24	64.43	56.65
7	78.26	79.24	72.43	72.18	68.04	74.93	72.23	61.48	69.18	67.22	66.72	59.18
8	82.24	84.34	79.44	75.82	72.84	76.42	73.28	68.32	72.34	70.34	69.44	64.82

Table 4. Stability of optimized Voriconazole niosome formulation under refrigeration temperature (2-8°C) storage condition

Time (days)	Entrapment efficiency (%)	Drug remaining (%)
0	84.50	100.00
7	84.36	83.00
15	84.24	68.00
30	83.91	99.30
45	83.39	69.00
60	81.55	96.50
90	77.61	91.84

Table 5. A comparative study of anti-fungal activity of voriconazole niosome against *Candida albicans* and *Aspergillus fumigates*

S.No.	Solution	Mean of diameter of zone of Inhibition (mm)
Candida albicans	Vozole (1 % w/v)	10.11
	VN2	26.01
	VN4	18.01
Aspergillus fumigates	Vozole (1 % w/v)	10.17
	VN2	16.01
	VN4	14.05

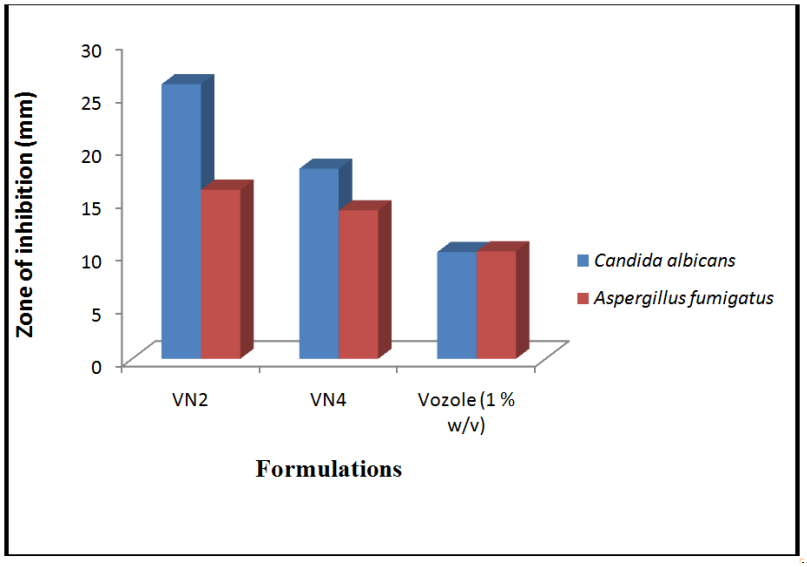

Figure 3. Zone of inhibition of (VN2), (VN4) and marketed (Vozole%w/v) eye drop

In other hands Tween 80 containing higher HLB value & smaller the alkyl chain & Brijj 30 containing higher HLB value & smaller lauryl chain length causes lower incorporation of the drug in vesicular system produce less amount of drug release which depicited in table no 3 (VN5 to VN12). (Darwish, 1998) Apart from that the nature of surfactant & lipid matrix charge inducer also play an important role in drug release pattern in vesicular system.with evidence of above sentence the DCP has negative charge inducer produce effect on providing integrity, uniformity, percent aggregration & fusion of noisome which

end of three months. The results concluded that almost 99% of the drug was retained upto 1 month and at the end of the study 91.84% drug was retained by the formulation (Table no 4).

Antifungal activity

The microbiological assays were carried out to inhibit the growth of *Candida albicans* and *Aspergillus fumigates* by using agar diffusion method the marketed formulation was Vozole 1% w/v showed least values as compared to both optimized formulation VN2 (Control I) &VN4 (control 2)

against *Aspergillus fumigatus* than *Candida albicans*.The zone of produced by optimized formulation with DCP show maximum that is 26.01mm, 16.0 mm & without DCP show 16.01mm, 14.05mm for *Candida albicans* and *Aspergillus fumigatus*. The data reveals that due to presence of charge on DCP may be contribute to higher penetration as well as higher diffusion in the study. In other hand the result of marketed formulation in terms of fungal growth inhibition produced less value which directly indicates the absence of DCP & optimized lipid matrix membrane in the formulation.

Conclusion

The investigation conclusively supported niosomes to be an advantageous drug delivery system. The study also conclude that cholesterol content, type of surfactant and the presence of charge inducer dicetyl phosphate (DCP), altered the entrapment efficiency EE% and release rate from voriconazole niosomes. Voriconazol based niosome containing span 60 with optimize ratio of lipid component with DCP (VN2) provide most beneficial for the highest entrapment & maximum drug release as compare to other formulations. Niosome may be considered as promising ophthalmic carriers for the topical application of Voricanazole.

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