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Development and Characterization of Voriconazole loaded Solid Lipid Nanoparticle for Topical Drug Delivery.

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ABSTRACT

Voriconazole is one of the second generation antibiotics which are synthetic triazole with improved anti-fungal activity. Currently voriconazole is available commercially as intravenous and oral formulations. The marketed formulations are associated with the hepatic and visual abnormalities. Voriconazole is also playing a major role in the treatment of topical disease like aspergillosis, candidasis etc. The present study is to assess the role of solid lipid nanoparticles in improving the therapeutic efficacy of voriconazole. In the present research solid lipid nanoparticles were prepared by the high shear homogenization method using compritol ATO 888, stearic acid and tristearine as lipids, Polaxomer 188 as surfactant and soya lecithin as co-surfactant. The prepared solid lipid nanoparticles were evaluated for their physicochemical properties like particle size, PDI, Entrapment efficiency, drug release study etc. Among all the 9 formulations the solid lipid nanoparticles prepared with stearic acid were found to have the good physicochemical properties. Solid lipid nano particles prepared with 300 mg of stearic acid found to have the lesser particle size (244.58±07.45) and good release in comparison with other formulations. The polydispersity index values were also found to have less than 0.5 for all the formulations which is an indication for uniform particle size. Voriconazole solid lipid nanoparticles prepared with 300 mg of stearic acid was found to have the controlled drug release. Solid lipid nanoparticles prepared with 300 mg of stearic acid was found to have the good physical stability up to 3 months.

Keywords: Voriconazole; Solid lipid nanoparticles, Topical drug delivery, Zeta potential

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INTRODUCTION

Fungi are present throughout the environment, human exposure is inevitable for fungal infections and are spreading very rapidly and estimated to occur in billion of people each year. These infections affect different parts of body including skin, nails, hair, scalp and eye [1]. Several treatment options are available for cutaneous fungal infections. A number of drugs such as miconazole, clotrimazole, Voriconazole (VOR) and ketoconazole have been used for the treatment of fungal and yeast infections [2]. Many antifungal agents are compounded in different types of excepients/vehicles and have been found to be effective [3].

The therapeutic difficulties stimulate constant search for improved formulations with low toxicity and high safety for treatment of fungal infections [4].

Voriconazole (VOR) is one of the potent antibiotics, which is a synthetic triazole with improved anti-fungal activity by causing cell lysis by inhibiting the 14- alpha lanosterol demethylation and also by preventing the production of ergosterol which is an essential constitute in fungal cell membrane [5]. Voriconazole (VOR) can be used in the treatment of esophagel candidiasis, invasive pulmonary aspergillosis [6] and also for the other fungal infections caused by *Scedosporium apiospermum* and *Fusarium species*.

VOR is poorly soluble in highly polar and apolar solvents, having limited oral bioavailability due to its low aqueous solubility and membrane permeability [7]. This is due to its structural features of VOR; currently available marketed formulations are having the side effects related to the visual and hepatic abnormalities [8]. Due to these challenges, lipid-based formulations of VOR were developed to decrease the cost of the treatment, to enhance the oral bioavailability of VOR and to reduce its toxicity [9]. Still proficient carrier-based formulation is needed with good therapeutic efficiency and with reduced toxicity.

Amongst the various nanocarriers studied so far, solid lipid nanoparticles are recently emerging as a novel approach to oral, parenteral, topical drug delivery systems [10]. In theory, solid lipid nanoparticles (SLN) combine the advantages of lipid emulsion systems and polymeric nanoparticle systems while overcoming the temporal and *in vivo* stability issues that plague the aforementioned approaches [11]. Utilizing biological lipids is theorized to minimize carrier cytotoxicity, and the solid state of the lipid is theorized to permit more controlled drug release with excellent safety and efficacy [12].

To circumvent the drawback associated with conventional delivery of drugs, lipid-based carrier of therapeutics products were used as delivery system [13]. The small size of lipid nanoparticles can able to enhance the dissolution profile and the lipidic carrier helps in permeating the drug through the biological barriers [14]. Furthermore, lipid nanoparticles are able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis. Advantages of using lipid as carrier systems for drug delivery are also related to their physiological nature, which reduces the risk of toxicological problems, local and systemic irritancy [15].

By taking all the advantages of lipid carriers for into account, Voriconazole loaded solid lipid nanoparticles (SLNs) were developed by employing high shear homogenization technique and the SLNs were characterized for PS, PDI, ZP and %EE [16]. Finally optimised formulation was characterised for release profile in various biological relevant fluids.

MATERIALS AND METHODS

Materials: Voriconazole was received as a gift sample from Hetero Labs Pvt. Ltd, stearic acid, compritol ATO 888, tristearine Pluronic®F-68, Tween-80, soya lecithin were supplied by Hi-Media Laboratories, Mumbai, India, and all other solvents used are of analytical grade.

Methods:

Preparation of Voriconazole loaded solid lipid nanoparticles:

Solid lipid nanoparticles for Voriconazole were prepared by using high shear homogenisation technique [17]. Briefly the drug and co-surfactant were dispersed in lipid with aid of heating and further mixing

of this lipid-drug solution to the preheated surfactant solution under high speed homogenization for few minutes and then subjected to ultrasonication by using probe sonicator in ice-bath [18]. The formula of Voriconazole loaded solid lipid nanoparticles were shown in table 1 along with the quantity of lipids and other excipients.

Table 1: Composition of Voriconazole loaded solid lipid nanoparticles

Batches	VOR-SLN1	VOR-SLN2	VOR-SLN3	VOR-SLN4	VOR-SLN5	VOR-SLN6	VOR-SLN7	VOR-SLN8	VOR-SLN9
Drug (mg)	30	30	30	30	30	30	30	30	30
Compritol ATO 888 (mg)	150	300	450	-	-	-	-	-	-
Stearic acid (mg)	-	-	-	150	300	450g	-	-	-
Tristearine (mg)	-	-	-	-	-	-	150	300	450
Polaxomer 188 (1% W/V) (mL)	50	50	50	50	50	50	50	50	50
Soya lecithin (mg)	70	70	70	70	70	70	70	70	70
Acetone (mL)	2	2	2	2	2	2	2	2	2

EVALUATION OF VOR-SLNs:

Particle size, Polydispersity index (PDI):

Intensity mean hydrodynamic size, polydispersity index (PDI), and zeta potential of the particles were measured on a Malvern Zetasizer-NanoZS with a He–Ne laser with a wavelength of 632.8 nm. The measurements were carried out at a scattering angle of 173° using disposable sizing cuvette and keeping the temperature at 25°C throughout the experiments. All measurements were repeated three times (n = 3) and the results are given as the effective diameter, also called Z-average diameter, and the PDI as a parameter of the particle size distribution [19]. The measurements were performed at 25°C, in triplicate (n =3) and the average values were calculated.

Zeta potential (ZP):

Zeta potential of solid lipid nanoparticles was measured at 25°C by using Zetasizer NanoZS (Malvern, UK).Water was used as dispersant and system was maintained at 25°C. Zeta potential was calculated from the Electrophoretic mobility using Helmholtz–Smoluchowski equation [20]. Each sample was measured in triplicate.

Total Drug Content (TDC)

The total drug content of VOR-SLNs was done by using in-house developed UV- spectroscopic method. Briefly 1 ml of VOR-SLNs was dissolved in methanol and final volume was made up to 10 ml and filtered through 0.45µm syringe filter. The resultant solutions were scanned by using UV- spectrophotometer by making proper dilutions and the TDC was calculated by using the following formula [21].

$$\textbf{Total drug content}(\%) = \frac{\textbf{concentration} * \textbf{dilution factor} * \textbf{volume of formulation}}{\textbf{Theoritical drug amount used}}$$

Entrapment Efficiency (EE):

The EE of VOR-SLNs was calculated by using the amount of free drug. Free drug concentration in VOR-SLNs was determined by reverse bag dialysis method. Briefly, a dialysis bag (regenerated cellulose membrane, molecular weight cut-off 12,000–14,000 Da) containing 1 ml solution of 1% w/v Poloxamer 188 in distilled water. Bag was dipped into 10 mL of VOR-SLNs. After equilibrium (24 h), dialysis bag was withdrawn from the

nanosuspension [22]. Sample collected from dialysis bag was assayed by UV spectroscopic method. Free drug, total drug content, entrapment efficiency and drug loading were calculated according to the following formula.

$$\%\mathbf{EE} = \frac{\mathbf{Wt} - \mathbf{Wf}}{\mathbf{Wt}} * \mathbf{100}$$

Wt and Wf are the total amount of drug in carriers and free drug respectively.

Scanning Electron Microscopy (SEM):

The external morphology of the prepared VOR-SLN4 and VOR-SLN5 was determined by using scanning electron microscope (JSM 6100 (JEOL), Japan). Samples were positioned on the stubs with double-sided adhesive tape and further sputtered with gold palladium alloy [23] (150–200A°). The electron microscope was functioned at an acceleration voltage of 20 KV. Working distance maintained at 12–14 mm. The samples were viewed at magnification of ×350 to x1500 with secondary electron (SED) and back-scattered electron detector (BSED) [24].

In vitro drug release studies:

Franz diffusion cell [25] with internal diameter 2.7 cm, and 40 mL capacity was used to determine the in-vitro release of prepared VOR-SLNs. 5 mL of VOR-SLN suspension was kept in donor chamber and 25 mL of pH 7.4 buffers with 1% benzalkonium chloride (BKC) as dissolution media and separated from dialysis membrane[25]. Franz diffusion cell was maintained at 37±1°C and stirred at 300 rpm. The sink condition was maintained throughout the release studies. Aliquots of sample were collected at particular intervals and the same amount of buffer was replaced with fresh medium [26]. Aliquots of samples were filtered with 0.2 µm and the samples were analysed with spectrophotometer at 256 nm. Release kinetics was determined from the invitro drug release data fitted to Higuchi and Korsmeyer-peppas equations [27].

Physical stability of VOR-SLNs:

Storage stability of optimised VOR-SLNs was performed by storing the samples [28] at 2-8°C, 25°C/60% RH and at 40°C/75% RH for 3 months. Periodically the samples were withdrawn and re-evaluated for physical parameters like particle size, PDI and zeta potential.

RESULTS AND DISCUSSION

Voriconazole SLNs were prepared by the high shear homogenization technique by using different lipids to monitor the effect of different lipids on VOR-SLNs physicochemical parameters. In the current research work three lipids were selected based on the drug solubility. Each lipid was used in three different ratios to monitor its effect on the VOR-SLNs.

Particle size, Polydispersity index (PDI):

Particle size and PDI are the resultant of the concentration of lipid, surfactant and co-surfactant [29]. Particle size and PDI of the prepared voriconazole loaded solid lipid nanoparticles was determined using the Malvern particle size analyzer [30]. Particle size and PDI for the prepared voriconazole loaded solid lipid nanoparticles was shown in table 2. All the particle size analysis was made in triplicate for accuracy.

Among all the VOR-SLNs, VOR-SLN4 and VOR-SLN5 were found to have the fewer particles size i.e. 300 ± 04 nm, 244±07 nm, and PDI 0.221± 0.1, 0.237±0.1 respectively. Both VOR-SLN4 and VOR-SLN5 were made with stearic acid as a lipid in 1:5 and 1:10 ratios respectively.

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Table 2: Particle size and polydispersity index of VOR-SLNs

S. No	Batch Code	Particle size (nm± SD)	PDI (± SD)
1	VOR-SLN1	597.12±22.41	0.451±0.6
2	VOR-SLN2	434.18±05.18	0.323±0.2
3	VOR-SLN3	602.61±12.27	0.439±0.2
4	VOR-SLN4	300.12± 04.89	0.221± 0.1
5	VOR-SLN5	244.58±07.45	0.237±0.1
6	VOR-SLN6	464.02±11.71	0.338±0.09
7	VOR-SLN7	312.62±08.25	0.507±0.8
8	VOR-SLN8	417.47±05.88	0.339±0.03
9	VOR-SLN9	552.94±11.72	0.218±0.02

Zeta potential (ZP):

Zeta potential plays an important role in the prediction of stability of the solid lipid nanoparticles during short and long term storage. Higher values of ZP more than ±25mV can able to stabilize the SLN suspension by causing the electric repulsion. Electric repulsion generally leads to minimal contact and aggregation between the particles. Particles charge can also result in interaction with tissues and cells [31]. Ionic surfactants and charge inducers can be utilized in the formulation to induced both positive and negative values of ZP. Dynamic light scattering and Laser Doppler are the examples of techniques to measure zeta potential. pH, ionic strength and the type of suspension are also the factors that affecting zeta potential of the formulation [32].

Zeta potential of the prepared suspensions was shown in table 3. All the prepared formulations were found to have negative zeta potential in the range of -17.8 to -38.5mV. Formulations made with stearic acid were found to have higher zeta potential values in comparison with other lipids. It was proved that the value of zeta potential was increasing upon increasing the lipid concentration.

Table 3: Zeta potential of VOR-SLNs

S. No	Batch Code	Zeta potential (mV± SD)
1	VOR-SLN1	-17.8±2.3
2	VOR-SLN2	-21.6±1.5
3	VOR-SLN3	-27.9± 0.62
4	VOR-SLN4	-21.3± 1.4
5	VOR-SLN5	-38.5±1.2
6	VOR-SLN6	-32.1± 0.37
7	VOR-SLN7	-21.9± 0.2
8	VOR-SLN8	-28.58± 0.8
9	VOR-SLN9	-25.65± 0.5

Total Drug Content (TDC) and Entrapment efficiency:

Total drug content of all the prepared batches were found to be in the range of 82 to 97 % which indicates no significant loss of drug during preparation of SLNs. Entrapment efficiency of prepared VOR-SLNs was in the range of 52 to 98 %. VOR-SLN9 showed the least entrapment efficiency i.e. 52.28% ±5.3 and the VOR-SLN5 shown the higher entrapment efficiency i.e. 97.89%±2.7. This is may be due to the higher affinity of the drug towards stearic acid. Among the three ratios 1:10 was found to be the optimum for the preparation of solid lipid nanoparticles.

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Table 4: Total drug content and Entrapment efficiency of VOR-SLNs

S. No	Batch Code	Total drug content (%± SD)	Entrapment efficiency (%± SD)
1	VOR-SLN1	82.26±3.8	65.12±2.8
2	VOR-SLN2	85.18±5.4	72.85±1.5
3	VOR-SLN3	90.27±1.7	65.59±8.1
4	VOR-SLN4	88.16±3.6	68.32±3.9
5	VOR-SLN5	96.63± 1.7	97.89± 2.7
6	VOR-SLN6	89.59± 4.1	86.16± 6.2
7	VOR-SLN7	78.36± 5.4	78.54± 3.7
8	VOR-SLN8	82.57± 2.6	82.51±2.6
9	VOR-SLN9	82.19±2.9	52.28±5.3

Scanning Electron Microscopy (SEM):

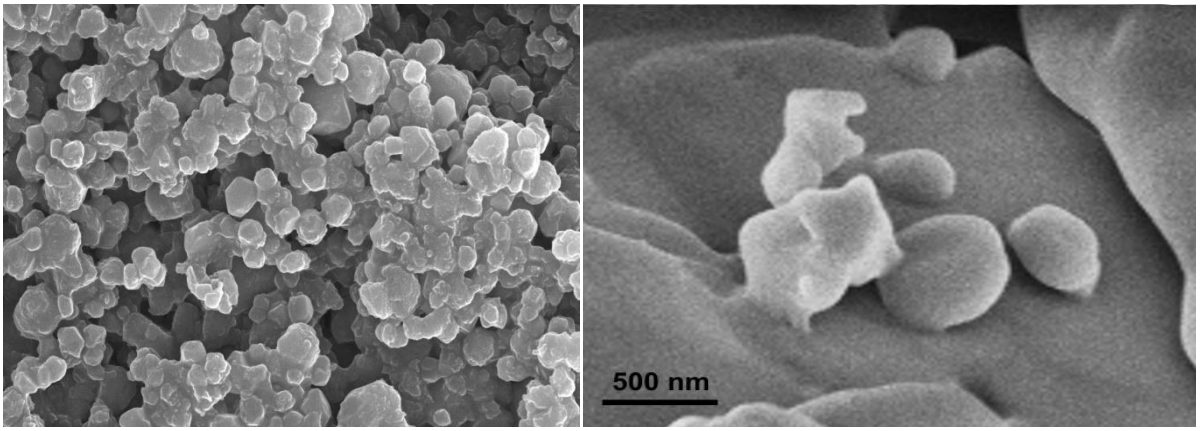

Fig 1: SEM Images of VOR-SLN5

In vitro drug release studies:

In-vitro drug release studies was performed for the prepared VOR-SLNs with franz diffusion cell using pH 7.4 buffer with 1%v/v BKC as medium.1% v/v BKC used as surfactant to increase the solubility of voriconazole in medium to maintain sink condition during the release study. Voriconazole solid lipid nanoparticles shown sustained release patterns, but the release kinetics of the VOR-SLNs are changed which may be due to the usage of different lipids in preparation of solid lipid nanoparticles. The release data was fitted into different kinetic models to like zero order, first order, Higuchi, Hixon- crowell and korsmeyer-peppas model to predict the release mechanism and the regression values (R2). The sustained release pattern of VOR-SLNs may be due to the entrapment of drug in the lipid core matrix. Drug release data and drug release kinetics for the prepared VOR-SLNs was calculated and the values were shown in table 5 and table 6 respectively. Among all the formulations it was found that the VOR-SLN5 is having the desired drug release profile in comparison with other formulations. The release data of VOR-SLN5 was best fitted into Higuchi model with R2 values 0.960.

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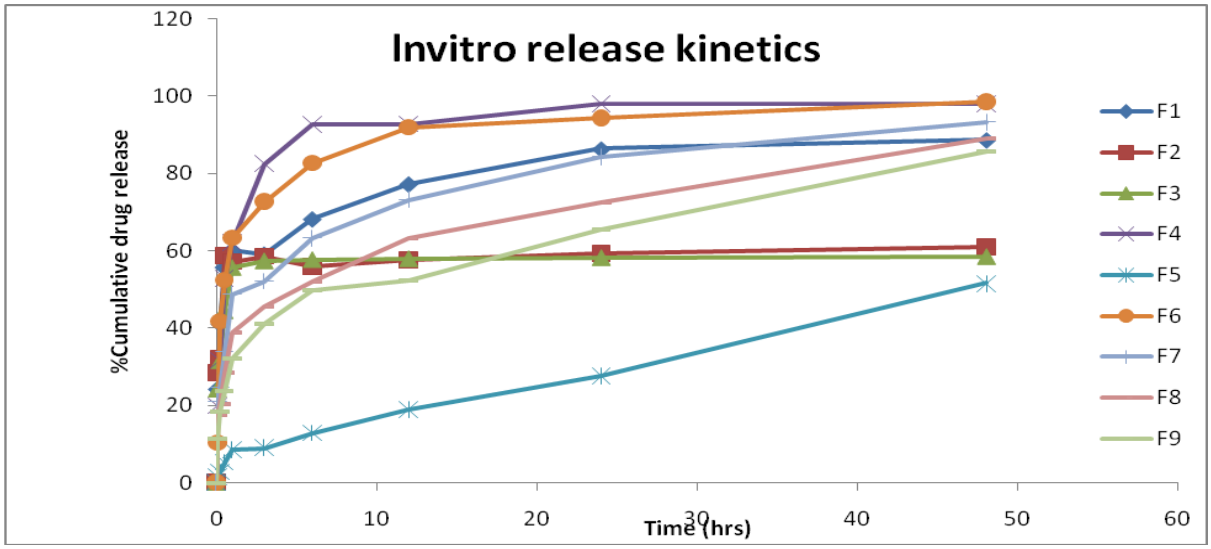

Fig 2: Invitro drug release profile of Voriconazole SLNs prepared by using different lipids.

Table 5: Invitro release data of VOR-SLNs:

	%DRUG RELEASED								
TIME (HRS)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.08	24.13	28.39	24.13	19.81	1.45	10.26	21.07	17.56	11.52
0.25	41.03	32.08	31.51	31.62	2.79	41.78	24.71	20.31	18.56
0.5	55.73	58.83	44.44	52.81	5.23	52.43	33.76	28.64	23.85
1	60.2	57.21	55.65	62.19	8.56	63.44	48.72	38.95	32.16
3	59.06	58.64	57.35	82.58	9.05	72.76	52.09	45.65	41.25
6	68.17	55.94	57.65	92.76	12.89	82.77	63.21	52.16	49.86
12	77.23	57.65	57.92	92.89	18.87	92.09	73.23	63.18	52.31
24	86.29	59.36	58.19	98.02	27.63	94.41	84.25	72.45	65.48
48	88.65	61.07	58.46	98.15	51.68	98.73	93.27	88.97	85.64

Table 6: Invitro release kinetics of VOR-SLNs:

Batches	Zero order model	First order model	Higuchi model	Korsmeyer- peppas model	
	R^2	R^2	R^2	R^2	n
VOR-SLN1	0.434	0.147	0.677	0.154	0.255
VOR-SLN2	0.181	0.089	0.347	0.088	0.182
VOR-SLN3	0.210	0.090	0.413	0.113	0.205
VOR-SLN4	0.383	0.162	0.641	0.223	0.323
VOR-SLN5	0.974	0.607	0.960	0.782	0.544
VOR-SLN6	0.421	0.172	0.668	0.260	0.358
VOR-SLN7	0.626	0.219	0.849	0.241	0.316
VOR-SLN8	0.711	0.253	0.903	0.274	0.328
VOR-SLN9	0.755	0.288	0.920	0.336	0.361

Physical stability study of VOR-SLNs:

Table 7: Physical stability of VOR-SLNs:

Batches	Condition	Particle size (nm)	PDI	Zeta potential (mV)
VOR-SLN5	**Initial**	244.58±7.45	0.237±0.10	-38.52±1.20
	2-8°C (1M)	262.48± 4.70	0.269±0.14	-35.17±0.30
	2-8°C (3M)	277.85± 2.18	0.310±0.29	-30.12±0.70
	25°C/60% RH (1M)	285.15± 3.90	0.343±0.27	-30.87± 0.63
	25°C/60% RH (3M)	300.67± 4.67	0.433±0.85	-28.72± 0.34
	40°C/75% RH (1M)	309.12± 2.85	0.523±0.19	-27.81± 0.70
	40°C/75% RH (3M)	350.98± 5.65	0.623±0.73	-25.63± 0.56

CONCLUSION

Current research indicating that the lipophilic drugs like voriconazole can also formulated as solid lipid nanoparticles by selecting proper lipid, surfactant and co-surfactant in proper ratios. The present research helps in finding the suitable formula for the preparation of stable voriconazole solid lipid nanoparticles with desired particle size, PDI and other parameters. Among all the three lipids it was concluded that stearic acid is the potential lipid to formulate voriconazole loaded solid lipid nanoparticles. This is may be due to the molecular level of interaction between the lipid and drug. In the current study it w also concluded that the % entrapment efficiency will get improved with increase in amount of lipid up to certain limit. Zeta potential of the prepared formulations also played a major role in selecting the final formula for the formulation of voriconazole loaded solid lipid nanoparticles. The negative zeta potential helps in predicting the storage stability. The solid lipid nanoparticles obtained in the range of 200 to 400 nm with higher zeta potential are selected as the optimum formulations for proceeding to further investigation studies. From the invitro release studies it was indicated that the VOR-SLN5 is following the zero order release with R2 value 0.974. It is indicating that the VOR-SLN5 is following the Higuchi model with coefficient of regression value 0.960 and from the n value 0.544 of korsemeyer- peppas model it is indicating that the VOR-SLN5 is following the anomalous diffusion which indicates that the release pattern is occurring by both the dissolution as well as diffusion. Short term stability studies data indicating that the VOR-SLN5 is physically stable for 3 months at all the stability conditions.

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