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VORICONAZOLE SOLID LIPID NANOPARTICLES: OPTIMIZATION OF FORMULATION AND PROCESS PARAMETERS

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ABSTRACT

Colloidal drug delivery system is an emerging area that has been contributing appreciably to the progress in the field of controlled as well as targeted drug delivery. Solid lipid nanoparticles (SLNs) by their composition are best suited for drug delivery through the skin for both topical and systemic action. The objective of this study was to develop stable solid lipid nanoparticles of selected model drug i.e. Voriconazole with improved characteristics like high entrapment efficiency and less particle size so that they can be effectively administered by topical route. In this present study, SLNs of Voriconazole were prepared by two methods viz. Emulsion solvent evaporation method and modified hot homogenization method with three different formulation variables in each method that were type of lipid (Cetostearyl alcohol and Imwitor 491), drug-lipid ratio (1:2.5, 1:5 and 1:7.5) and concentration of surfactant tween 20 (0% and 0.5% v/v). The entrapment efficiency was found to be influenced by all the variables of the study and the SLNs prepared with Imwitor 491 at 1:7.5 ratio by emulsion solvent evaporation in presence of surfactant was found to have maximum entrapment efficiency i.e. 84.24%. The particle size and surface morphology were determined by scanning electron microscopy and all formulations of SLNs were found to be in the size range of 207 – 312 nm. These results were subjected to ANOVA studies. The size of SLNs prepared from modified hot homogenization method was found to be small when compared to those prepared by emulsion solvent evaporation method.

***Key words:** Solid lipid nanoparticles, Voriconazole, Imwitor, Entrapment efficiency, Particle size*

INTRODUCTION

Colloidal carriers are one of the approaches for the controlled delivery of drugs by the dermal route. As a vehicle for controlled release of active substances and targeting to skin layers, nanodisperse systems such as liposomes, nanoemulsions, and lipid nanoparticles are gaining significant importance. Among the different colloidal carriers in this context, Solid lipid nanoparticles

(SLNs) by their composition are best suited for drug delivery through the skin for both topical and systemic action [1]. SLNs are developed from lipids which have a structure similar to phospholipids in the biological membranes and these SLNs impart lipophilicity to the incorporated drugs so that their permeability is improved. The lipids employed are biocompatible, biodegradable and also nontoxic so that the SLNs are superior to polymeric nanoparticles [2]. Besides improving skin permeability, these can also improve the bioavailability of drugs through any route. The SLNs can diffuse deeper into the tissues because of their size which is 50 – 1000nm, so that these can also be used in delivering the drugs to the desired tissue by both passive as well as active targeting approaches [3].

SLNs are the best dosage forms for drugs that have limited bioavailability because of poor permeability. The drugs with poor lipophilicity have poor permeability as the later is a function of former. The major objective of this study was to develop stable solid lipid nanoparticles with improved characteristics like high entrapment efficiency and less particle size for selected model drug, voriconazole which is having poor lipophilicity. In this work, the influence of various process and formulation parameters on entrapment efficiency and particle size were studied. The process parameter selected was the method of preparation; SLNs of voriconazole were prepared by two methods viz. emulsion solvent evaporation method and modified hot homogenization method. In each method three formulation variables were considered viz. type of lipid, drug-lipid ratio and surfactant concentration. Two lipids, Cetostearyl alcohol (CSA) and Imwitor 491 (Glyceryl Stearate) were employed in this study. These lipids were taken at three different ratios with a drug that were 1:2.5, 1:5 and 1:7.5. All these formulations were prepared without surfactant and with a surfactant, polysorbate 80 at 0.5% v/v in the aqueous phase.

MATERIALS AND METHODS

Materials

Voriconazole and Imwitor 491 were obtained as gift samples from Hetero labs Ltd.; Ceto stearyl alcohol was obtained from Loba Chemie Pvt Ltd, Mumbai; Chloroform was obtained from Merck Specialities Pvt. Ltd. All other materials used were of analytical grade.

Methods

Fourier Transform Infrared Spectroscopy (FT-IR)

The physicochemical compatibility of Voriconazole with CSA and Imwitor were studied by FT-IR studies. Pure drug and physical mixtures of the drug and polymers were prepared as pellets along with KBr in a hydraulic press. The spectra were recorded in the wavelength region of 4000 cm^{-1} to 400 cm^{-1} by subjecting the pellets to Bruker's Fourier Transform Infrared Spectrophotometer [4]**.**

Preparation of Solid Lipid Nanoparticles of Voriconazole

Voriconazole Solid Lipid Nanoparticles were prepared by two methods with two CSA and Imwitor, and the formulation compositions were shown in Tables 1 & 2.

Modified hot homogenization method

This method was a modification of the method reported by Nisha Raina *et al* [5]. Here, the lipid was heated to its melting point; the drug was dissolved in it to form the oily phase and was maintained at the temperature. Simultaneously aqueous phase containing distilled water without or with 0.5% v/v polysorbate 80 was also heated to the same temperature as that of the oil phase. Both the phases were subjected to vortex mixing to form a pre-emulsion by maintaining the same temperature. The above formed pre-emulsion was poured into ice cold water under high speed stirring to form an immediate nanoemulsion which was further solidified to give a suspension of solid lipid nanoparticles.

Solvent evaporation method

The method adopted here was a modification of double emulsion solvent evaporation method reported by Sanyog Jain *et al* [6]. Weighed quantities of drug and lipid were dissolved in chloroform to form an organic phase. The aqueous phase containing distilled water without or with 0.5% v/v polysorbate 80 was dropped into the above organic phase to form primary w/o emulsion. Primary emulsion was added to 15 ml water. The emulsion was subjected to rapid stirring at 1200 rpm until the Chloroform was evaporated completely.

Table 1: Formulations of Voriconazole SLNs with lipid CSA

S. No.	Surfactant Concentration (0.5% v/v)	Melt dispersion			Solvent evaporation		
		Drug : Lipid ratio			Drug : Lipid ratio		
		1:2.5	1:5	1:7.5	1:2.5	1:5	1:7.5
1	0	F1	F2	F3	F7	F8	F9
2	0.5	F4	F5	F6	F10	F11	F12

Table 2: Formulations of Voriconazole SLNs with lipid Imwitor 491

S. No.	Surfactant Concentration (0.5% v/v)	Melt dispersion			Solvent evaporation		
		Drug : Lipid ratio			Drug : Lipid ratio		
		1:2.5	1:5	1:7.5	1:2.5	1:5	1:7.5
1	0	F13	F14	F15	F19	F20	F21
2	0.5	F16	F17	F18	F22	F23	F24

Characterization of SLNs

Measurement of particle size and surface morphology

SEM was used as a tool to determine particle size and surface morphology [7]. The samples for SEM were prepared by sprinkling the SLNs on a double adhesive tape, and then it was fixed on an aluminum stub. All samples were examined under a scanning electron microscope at an acceleration voltage of 30 kV [8].

Determination drug entrapment efficiency

A measured volume of a suspension of SLNs was taken and subjected to centrifugation at 12,000 rpm for 20 min. The supernatant from was collected and subjected to analysis by UV-Visible spectrophotometer to estimate the amount of drug present unentrapped [8]. The entrapment efficiency was calculated according to the following formula

$$\text{Drug Entrapment Efficiency (\%w/w)} = \frac{\text{Total amount of drug} - \text{Amount of drug in supernatant}}{\text{Total amount of drug}} \text{ x } 100$$

Drug loading efficiency

Drug loading capacity (DL) was calculated as drug entrapped in the SLNs versus the total amount of the drug and the lipids added during preparation, according to the following formula

$$\text{Drug Loading Efficiency} = \frac{\text{Amount of drug loaded in SLN}}{\text{Total amount of drug and lipids}} \text{ x } 100$$

In-vitro drug release

In vitro drug release could was studied by dialysis bag method. The suspension of SLNs was subjected to centrifugation to collect the pellet. The pellet was placed in the pre-soaked gelatine tubing and was tied at both ends. Then this gelatine bag was immersed in 100 mL of 0.1N HCl buffer medium taken in a beaker which was placed on a magnetic stirrer set up at 100 rpm. Samples were taken periodically and subjected to spectrophotometrical analysis to determine the amount of drug released.

Drug release kinetics

The obtained drug release data was subjected to zero-order and first-order kinetic models to determine the order of kinetics and also subjected to Higuchi's and Korsmeyer-Peppas plots to determine the mechanism of drug release from the SLNs.

ANOVA studies

The results of entrapment efficiency and particle size were subjected to ANOVA studies to identify whether the selected process and formulation variables have significant influence or not individually and combined at levels taken in this study. The ANOVA studies were performed by Design Expert software [9].

RESULTS AND DISCUSSION

Compatibility Studies

The FT-IR spectra obtained were shown in Figure 1-3. The characteristic peaks of voriconazole obtained in the spectra of the pure drug were also observed at the same wavelength region in the spectra of physical mixtures of the drug with CSA and Imwitor 491. These results indicated that voriconazole was compatible with the selected lipids.

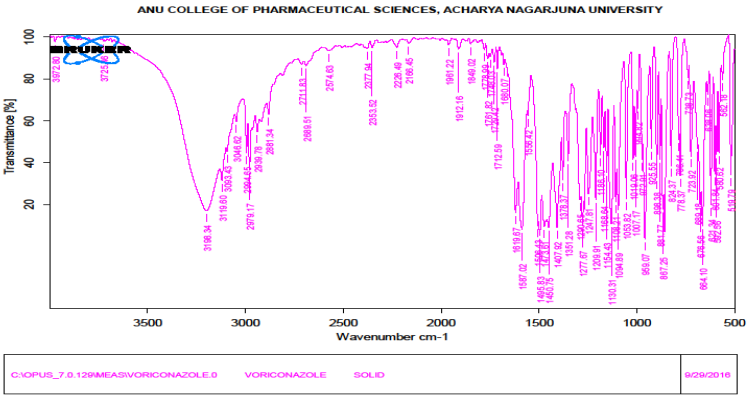

Figure 1: FT-IR spectra of Voriconazole

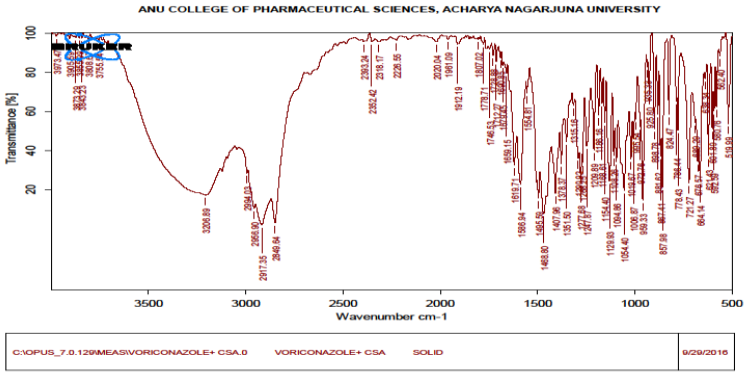

Figure 2: FT-IR spectra of physical mixture of Voriconazole and CSA

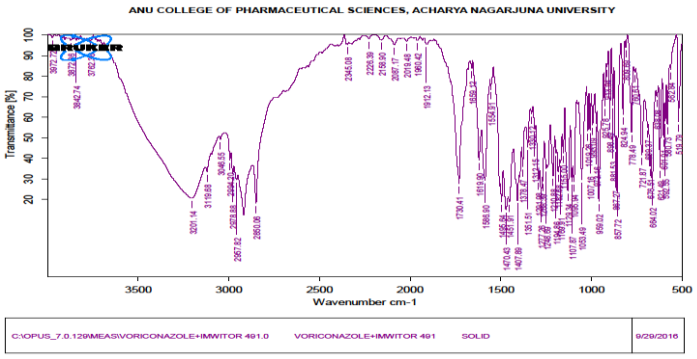

Figure 3: FT-IR spectra of physical mixture of Voriconazole and Imwitor 491.

Particle Size and Surface Morphology

The results of the particle size obtained from SEM analysis of all formulations were shown in Table 3. These results indicated that upon an increase in the amount of lipid in the formulation, the size was found to be increased (for example, a set of formulations of F1 to F3); this might be because of the deposition of more amount of lipid on the particle surface. The particle size was also found to be affected by the surfactant. The size of SLNs of formulations containing surfactant was found to be less than the size of SLNs of formulations without surfactant. This might be attributed to the emulsifying and stabilizing action of the surfactant. During the addition of oil phase to the aqueous phase, the surfactant might play a significant role in the formation of an emulsion with more stability and small particle size and as this nanoemulsion was developed into nanosuspension either by solvent evaporation or by cooling, the small globules were converted into small particles. But in the case of the formulations without surfactant, the stability might be poor and aggregation of particles might result which lead to relatively bigger size particles. The particle size was also influenced by the method of preparation of SLNs. It was found that the size of SLNs prepared from modified hot homogenization method was to be smaller than the size of SLNs prepared from solvent evaporation method. During the evaporation of chloroform in emulsion solvent evaporation method, the emulsion globules might be aggregated which resulted in the bigger size of SLNs upon complete evaporation of the solvent. The size of SLNs was also found to be influenced by the type of lipid and the size of SLNs prepared with Imwitor was found to be smaller than the size of SLNs prepared with CSA. This might be because of superior emulsifying and dispersing abilities of Imwitor than those of CSA.

The surface morphology of the SLNs (shown in Figure 4) was found to be smooth and uniform; this might be because of the texture of the lipids employed.

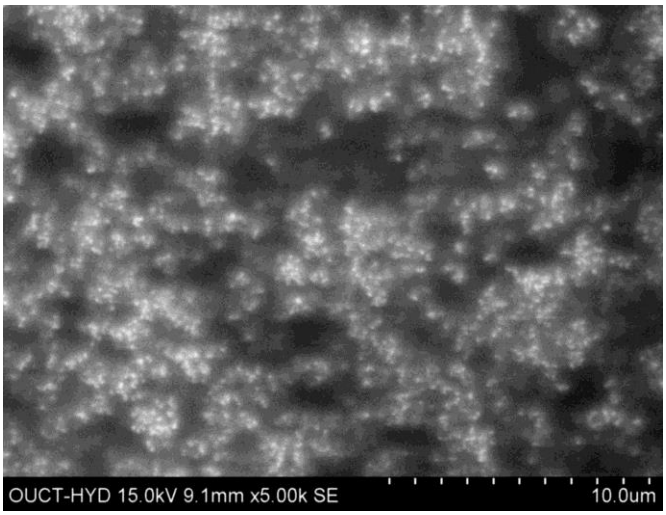

Figure 4: SEM image of Voriconazole SLNs of formulation F21

Table 3: Results Particle size, Entrapment efficiency and Drug loading of Voriconazole SLNs of all formulations

S. No.	Formulation	Particle size (nm)	Entrapment efficiency (%)	Drug loading (%)
1	F1	256 ± 1.6	34.03 ± 0.51	9.70 ± 0.11
2	F2	291 ± 2.1	48.13 ± 0.67	8.18 ± 0.13
3	F3	312 ± 2.2	54.81 ± 1.03	6.45 ± 0.12
4	F4	231 ± 2.1	30.67 ± 0.54	8.76 ± 0.15
5	F5	255 ± 2.2	39.19 ± 0.71	6.66 ± 0.11
6	F6	281 ± 1.6	45.53 ± 0.82	5.35 ± 0.10
7	F7	265 ± 1.9	38.25 ± 0.71	10.93 ± 0.21
8	F8	291 ± 2.8	59.81 ± 0.82	10.17 ± 0.18
9	F9	315 ± 3.1	78.99 ± 1.25	9.29 ± 0.13
10	F10	252 ± 1.9	33.16 ± 0.52	9.47 ± 0.15

11	F11	267 ± 2.3	45.33 ± 0.81	7.71 ± 0.13
12	F12	291 ± 2.7	60.10 ± 1.08	7.07 ± 0.14
13	F13	218 ± 1.4	44.64 ± 0.57	12.75 ± 0.21
14	F14	233 ± 2.5	68.48 ± 1.21	11.64 ± 0.19
15	F15	248 ± 2.4	73.10 ± 0.95	8.60 ± 0.14
16	F16	207 ± 2.1	39.20 ± 0.61	11.2 ± 0.19
17	F17	216 ± 1.3	54.59 ± 0.89	9.28 ± 0.17
18	F18	221 ± 2.1	63.70 ± 1.24	7.49 ± 0.12
19	F19	243 ± 2.3	73.94 ± 1.16	21.12 ± 0.32
20	F20	289 ± 2.7	80.00 ± 1.52	13.6 ± 0.24
21	F21	297 ± 1.7	84.24 ± 1.44	9.91 ± 0.17
22	F22	212 ± 1.9	48.15 ± 0.92	13.76 ± 0.24
23	F23	236 ± 2.2	60.13 ± 1.03	10.22 ± 0.18
24	F24	271 ± 1.9	68.32 ± 1.21	8.03 ± 0.16

Entrapment Efficiency

The EE was found to be influenced by all the formulation and process parameters and the results were shown in Table 3. Upon an increase in the amount of lipid, the EE was found to be increased [10] which might be ascribed to the fact that higher amount of lipids can hold a high amount of drug and also can prevent leakage of the drug during and after emulsification. The EE was found to be influenced by the surfactant also in both the methods as the EE was less in SLNs prepared with surfactant at 0.5% v/v level than the EE of SLNs prepared without surfactant. This might be because the surfactant acts as a bridge between both the phases at their interface and this might cause leakage of drug from the oil globules. The EE in the case of SLNs prepared from Imwitor 491 was found to be high than that of SLNs prepared from CSA this might be attributed to the characteristics of Imwitor viz. binding and retarding ability which might hold the drug when added to it and also might reduce the leakage further. The EE was found to be high in SLNs prepared by emulsion solvent evaporation than the EE of SLNs prepared from hot homogenization technique. In the later method, as the drug was directly dissolved in the molten lipid, it might be partitioned into aqueous phase but in the former method, the drug was combined dissolved and also combined precipitated along with the lipid from chloroform, its partitioning might be lesser.

Drug Loading

Drug loading is generally decreased upon an increase in the amount of lipid as evident from the formula of drug loading and the same was observed in this study also. The influence of other parameters on the drug loading was in the same order as that on the EE.

Drug Release Studies

The formulations of SLNs, F9 and F21 which showed high entrapment efficiency in each case of two lipids were subjected to drug release studies by dialysis bag method. The results were shown in Fig 5. The drug release rate was found to be more in F21 than that in F9. This might be because of the amphiphilic as well as solubilizing abilities of Imwitor 491 which made the drug release at a relatively faster rate than that from the SLNs prepared from CSA. Though the release rate was high in F21, it was sustained for a period of 12 h. These studies indicated that optimum drug release rate could be achieved from SLNs prepared from Imwitor 491.

Kinetics of drug release

The drug release from both the formulations was found to follow first – order kinetics and the mechanism of drug release was found to be Fickian diffusion as identified from the Higuchi's regression value and Korsmeyer-Peppas 'n' value as shown in Table 4. This might be because of the controlled drug diffusion from the spherical SLNs made from water insoluble lipids.

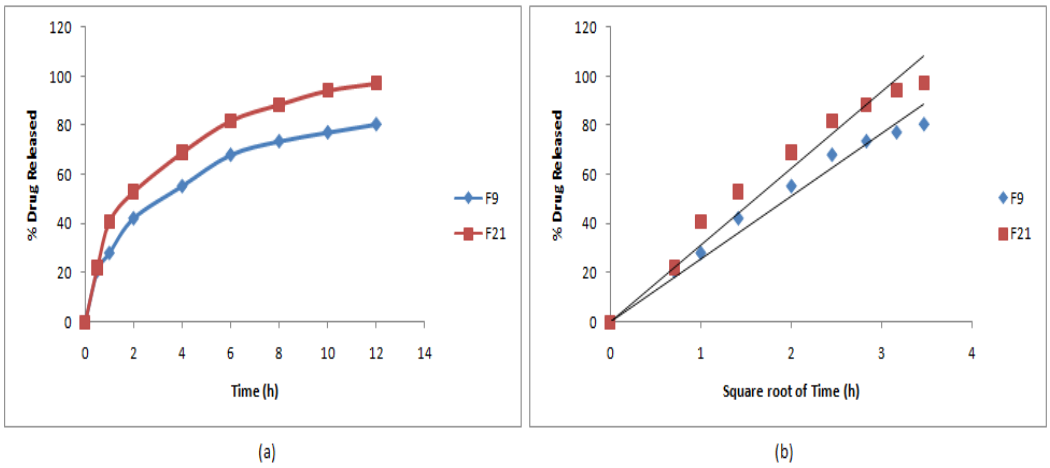

Figure 5: a) Drug release profile and b) Higuchi's plots of Voriconazole formulations F9 and F21

Table 4: Drug release kinetics of Voriconazole formulations F9 and F21

S. No.	Formulation	Regression coefficients			Peppas 'n' value	Drug release rate constant (h^{-1})
		Zero-order	First-order	Higuchi's		
1	F1	0.597	0.907	0.972	0.43	0.154
2	F2	0.544	0.989	0.958	0.44	0.290

ANOVA Studies

The results of the ANOVA studies (shown in Table 5) indicated that the whole experiment involved one process parameter and three formulation parameters were significant with respective to their influence on both particle size and entrapment efficiency. The influence of all the four parameters on particle size as well as entrapment efficiency was found to be significant at 99.5% confidence interval. In the case of Entrapment efficiency the combinations surfactant concentration with of each of the other three parameters viz. type of lipid, method of preparation and Drug-Lipid ratio, were found to be significant. But in the case of particle size, the combined influence of the type of lipid and method of preparation was found to be more significant than any other combination of factors. These ANOVA studies indicated that entrapment efficiency was a more sensitive parameter than particle size.

Table 5: Results of ANOVA studies for Entrapment efficiency and Particle size

Source of variation	Degrees of freedom		Error degrees of freedom		F value		p-value Prob>F	
	Entrapment efficiency	Particle size	Entrapment efficiency	Particle size	Entrapment efficiency	Particle size	Entrapment efficiency	Particle size
Whole-plot	11	11	2.00	2.00	835.07	309.98	0.0012	0.0032
A-Surfactant conc	1	1	2.00	2.00	1864.63	688.07	0.0005	0.0015
B-Lipid	1	1	2.00	2.00	3024.14	1206.76	0.0003	0.0008
C-Method	1	1	2.00	2.00	1503.01	468.45	0.0007	0.0021
D-Drug:Lipid	2	2	2.00	2.00	2291.06	668.84	0.0004	0.0015
AB	1	1	2.00	2.00	71.91	0.50	0.0136	0.5519
AC	1	1	2.00	2.00	198.92	2.92	0.0050	0.2294
AD	2	2	2.00	2.00	25.17	-5.743	0.0382	1.0000

						$x10^{-3}$		
ABC	1	1	2.00	2.00	18.90	47.35	0.0490	0.0205
ABD	2	2	2.00	2.00	38.51	-2.987 $x10^{-4}$	0.0253	1.0000
ACD	2	2	2.00	2.00	-1.639 $x10^{-3}$	-1.923 $x10^{-3}$	1.0000	1.0000
ABCD	2	2	2.00	2.00	31.85	8.84	0.0304	0.1017
BC	1	1	2.00	2.00	4.82	155.48	0.1594	0.0064
BD	2	2	2.00	2.00	33.98	-3.131 $x10^{-3}$	0.0286	1.0000
CD	2	2	2.00	2.00	-6.425 $x10^{-7}$	-5.467 $x10^{-3}$	1.0000	1.0000
BCD	2	2	2.00	2.00	194.01	43.88	0.0051	0.0223

Note: 1) Entrapment efficiency: Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, B, C, D, AB, AC, AD, BD, ABC, ABD, BCD, ABCD are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.
2) Particle size: Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, B, C, D, BC, ABC, BCD are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

CONCLUSION

The results of the present study showed that major characteristics of the SLNs, entrapment efficiency, and particle size were found to be influenced by many processes and formulation parameters. To achieve high entrapment efficiency of drugs in SLNs, formulations can be developed with emulsion solvent evaporation method with Imwitor as the lipid instead of hot homogenization method. This study also showed that the entrapment efficiency and particle size of SLNs would also depend on the amount surfactant in the formulation.

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