DESIGN AND CHARACTERIZATION OF ITRICONAZOLE LOADED SOLID LIPID NANOPARTICLES

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Abstract

The discovery of new drug molecules in the current era has come with incidences of low water solubility. The less solubility of drug in water decreases oral bioavailability of the drug molecules. According to biopharmaceutics classification system, the drugs are categorized as per their solubility and membrane permeability. BCS class II shows high membrane penetration with low water solubility. The low water solubility of drugs presents a problem in their release when given orally. Formulation of SLN (solid lipid nanoparticle) is one of the leading approach to improve the poor water solubility of the drug. Its ability to encapsulate the drug in lipid matrix offers a precursor in advanced level of drug targeting. Itriconazole is widely used anti-fungal drug of BCS class II. In this study, the Itriconazole containing solid lipid nanoparticles were formulated by three methods. The method optimization was done on entrapment efficiency, size of particles and yield. The optimization of variables was done using response surface method using 33 factorial designs. In this technique, three factors were investigated, at two different levels, and formulation trials were carried out over 27 possible combinations. The surfactant concentration (A), drug: lipid ratio (B), stabilizer concentration (C) were considered independent variables whereas the size of particles (X) and polydispersity index (Y) were considered as dependent variables. The in-vitro release investigation of drug containing solid lipid nanoparticles was compared with pure drug suspension where the solid lipid nanoparticles showed extended release of drug up to 24 hours with 89.47 %.

Keywords: solid lipid nanoparticles, BCS class II, Itriconazole, oral bioavailability, low water solubility.

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INTRODUCTION

The chemistry of lead compounds in drug discovery has increased the incidences of poor solubility of newly researched drug molecule. In the current scenario, roughly more than 60% of newly developed active pharmaceutical ingredients are not soluble in water or having very low aqueous solubility [1]. Presently, more than 40% market formulations used for oral administration are in the category of practically insoluble in water [2]. The compound which shows aqueous solubility less than 100 µg/ mL indicates low solubility and exists as rate limiting step in dissolution [3].

Biopharmaceutics classification system

The categorization of drugs as per Biopharmaceutics classification system (BCS) is based on their solvency and membrane permeability. It is considered to be prime criteria to resolve the issues related to drug molecules during drug development. The BCS category of drugs are following: high solvency/ high penetrability (class I), low solvency / high penetrability (class II), high solvency / low penetrability (class III), and low solvency / low penetrability (class IV) [4]. The drug included in BCS

class II shows high membrane permeability but have low water solubility as well. A large number of existing drug compounds belongs to that category like Itriconazole, cyclosporine, and itraconazole etc [5].The low water solubility of these drugs presents a great problem in their release when given orally. Such drugs show low oral bioavailability. By decreasing the size of particles and increasing the surface area might have significant effects on solubility profile of a drug molecule. Therefore, increasing in the drug solvency is viewed as a compelling strategy for enhancing the oral bioavailability of BCS class II drugs [6]. The changes in the crystal behaviour, comminution of the particles, pH alteration and self-emulsification are considered the useful techniques for improving dissolution profile of BCS class II drug [7].

Solid lipid nanoparticles

SLNs are nano-sized particles containing drug in lipid that persists in solid form at room and body temperature. They can be fabricated in very small size from 50 nm to 500 nm. Because of their smaller size, their surface area is increased and they exhibit high drug loading. They offer potential ability of encapsulating pharmaceuticals as well as nutraceuticals [8]. Solid lipid nanoparticles prepared from biodegradable lipids offers numerous advantages like controlled release, high drug loading, low toxicity and ease of large scale production [9].

MATERIAL AND METHODS

Itriconazole was procured as a gift sample from GSK Pharmaceutical Ltd. Mumbai. Different organic solvents including ethyl alcohol, methyl alcohol, acetone etc. were purchased from Merck chemicals Ltd. Mumbai. All reagents used in the research were of analytical category. Stearic acid, Gelucire, glyceryl monostearate (GMS), cetyl alcohol, Poly vinyl alcohol & Tween 80 were purchased from SD fine Limited, Mumbai.

Screening of lipids

The lipid selected for the screening were stearic acid, Gelucire, glyceryl monostearate (GMS) and cetyl alcohol. The vials were kept in sonicator at normal room temperature for 30 seconds. The lipid is supposed to be soluble when it disappears in the particular solvent on visual observation.

METHODS OF PREPARATION FOR SLNS

Three methods were used for the formulation of solid lipid nanoparticles.

Hot homogenization

In above method first the lipid was melted at a temperature above its melting point. The drug being lipophilic in nature was added in melted lipid. Another phase containing purified water mixed with surfactant was prepared and heated to the temperature similar to the lipid phase containing drug. Melted phase was dispersed in hot aqueous surfactant mixture drop wise and homogenised at high speed to make primary o/w type emulsion. This emulsion was again homogenized at high pressure to get solid lipid nanoparticles [10].

Cold homogenization

Cold homogenization was employed in the formulation of solid lipid nanoparticle with the target to avoid overheating in case of therolabile drugs. In this process, lipid was fist melted to a temperature higher than melting point and drug was added into the melted lipid. The drug loaded lipid phase was cooled and solidified by using dry ice or liquid nitrogen. The precipitated drug loaded solidified particles were coarse in size. The comminution of these particles was performed by using grinding mill to a size range of 50 to 100 µm [10-11].

Solvent emulsification-evaporation technique

In above method, the lipid was first melted over the melting point and drug was added into melted lipid. The lipid mixture containing drug was dissolved completely in an organic solvent by using sonication technique. The surfactant was dissolved in purified water to make an aqueous phase and the water phase was heated to the temperature similar to the lipid phase. Because of increased temperature, the volatile organic solvent gets evaporated and lipid nanoparticles start to precipitate. These lipid nanoparticles were solidified through cooling at room temperature and filtered through membrane filter [10-11].

METHOD OPTIMIZATION FOR FABRICATION OF SOLID LIPID NANOPARTICLES

The technique was optimized based on the particles size of the resultant nano lipid particles with polydispersity index (PDI). The technique through which lower particle size with high entrapment efficiency derived was selected for further progress in formulation development.

OPTIMIZATION OF FORMULATION VARIABLES

The optimization of variables employed in the formulation was performed using 3^3 factorial designs. In the present study, three independent variables were taken into consideration for the designing of formulation. The variables taken were the concentration of surfactant (A), drug: lipid concentration ratio (B) and concentration of stabilizer (C) in regression analysis. The dependent variables for the selection of optimized process and batch were considered the size of particles (X) and polydispersity index (Y). These were considered the parameters of importance for selection of appropriate combination of variables. Total 27 possible combinations were made and studies for designing of solid lipid nanoparticles. All the variables were examined at three different levels i.e. concentration of surfactant (4.0, 6.0 and 8.0 % v/v). The lipid concentration with respect to the drug taken as (5.0, 7.0 and 10.0 % w/w) and stabilizer was used in the concentration of (1.0, 2.0 and 3.0 % w/v).

SELECTION AND OPTIMIZATION OF PROCESS VARIABLES

The variables utilized in the formulation plays an important role in quality of desired formulation. The dependent variable for the present research were speed of homogenization, time of stirring, and temperature for getting SLNs. The individual effect of these variables was investigated in development of solid lipid nanoparticles. The response was recorded for particles size in nanometers and PDI.

Table 1: Response surface regression in different batches prepared for Itriconazole SLNs using 3^3 factorial designs

Formulation Code	Concentration of surfactant (%v/v)	Concentration of lipid (w/w %)	Concentration of stabiliser (w/v %)
GMS 1	4	5	3
GMS 2	6	5	3
GMS 3	8	5	3
GMS 4	6	7	1
GMS 5	8	7	1
GMS 6	4	7	1
GMS 7	8	10	2
GMS 8	4	10	2
GMS 9	4	5	1

GMS 10	6	7	3
GMS 11	8	7	3
GMS 12	4	10	1
GMS 13	4	5	2
GMS 14	8	7	2
GMS 15	6	10	3
GMS 16	6	7	2
GMS 17	8	10	3
GMS 18	4	7	2
GMS 19	8	10	1
GMS 20	6	5	1
GMS 21	4	10	3
GMS 22	6	5	2
GMS 23	6	10	2
GMS 24	4	7	3
GMS 25	6	10	1
GMS 26	8	5	1
GMS 27	8	5	2

FREEZE DRYING PROCESS FOR SOLID LIPID NANOPARTICLES

The prepared solid lipid nanoparticles were subjected to lyophilization using a freeze dryer. Lyophilization is done to improve the stability of developed SLNs. Initially, the samples were freeze dried at -40°C and subsequently temperature was increased up to 25°C with enhancement rate of 5°C per hour.

CHARACTERIZATION PARAMETERS FOR ITRICONAZOLE LOADED SOLID LIPID NANOPARTICLES

Entrapment efficiency and loading of drug

Entrapment efficiency and loading of drug were calculated using the formulas given below:

%EE = free drug amount / total weight of drug *100

% Drug loading= drug entrapped in SLNs/ weight of vehicle *100

Determination of yield of SLNs

Yield of the formulation indicates the quantity of solid lipid nanoparticles achieved after the preparation. The yield was calculated in percentage.

Analysis of particle size distribution

The particle analysis in solid lipid nanoparticles was investigated by photon correlation spectroscopy method.

Determination of zeta potential

The zeta potential studies for prepared formulations were carried out utilizing Zetasizer instrument.

Surface morphological studies by scanning electron microscopy (SEM)

The surface morphology is useful for study 3D structure of particles. The study was performed using scanning electron microscopy.

Investigation of In-Vitro Dissolution

The in-vitro release of drug from Itriconazole loaded SLNs was performed using dialysis membrane. The SLNs suspension in 1 ml quantity was poured to dialysis tube and sealed. The tube was transferred to a vessel having 10 ml of buffer solution pH
6.4 and 2% tween 80. The sample was subjected to a shaker apparatus maintained at 37±1°C. The speed of strokes was fixed at 5o min^{-1}. The samples in 2 ml quantity from the vial were taken out at time hour of 0, 0.5, 1, 2, 4, 8, 12, 16, 20 & 24h. The sink conditions were maintained by replacing the amount of sample with fresh media. The samples were analyzed by UV spectroscopy method at 291 nm. The drug release from SLNs was compared with the drug release from suspension of pure drug.

STABILITY STUDIES

The Itriconazole loaded SLNs were poured in 10 ml ampoule and sealed. The sealed ampoules were kept at temperature exposure of 5°C and 25°C for a time period of 6 months to evaluate stability of developed formulation. The particles were analyzed for changes in particle size, zeta potential and entrapment efficiency during the studies.

RESULTS AND DISCUSSION

Screening of the components revealed that the glyceryl monostearate (GMS) lipid would be appropriate in development of solid lipid nanoparticles. Ethanol as a solvent can dissolve both the drug and lipid, hence selected for formulation process of SLNs. Lipid matrix used in the formulation increase the drug solubility and subsequently the bioavailability of lipophilic drug.

Table 2: Mean particle size and %EE of SLNs (Mean±3, n=3)

Formulation technique	Mean particle size(nm)	%EE
Hot homogenization method	151.1 ±11.8	88.91 ±6.46
Cold homogenization method	314.6 ±15.1	69.71 ±5.68
Self-emulsification solvent-evaporation	781.3 ±18.5	53.20 ±4.42

The hot homogenization method was adopted for formulation of solid lipid nanoparticles on mean particle size & entrapment efficiency for further investigations.

The optimization of variables used in the formulation was done using surface response method. Response

surface method (RSM), statistically explore relative behavior of various variables used on the development of formulation. The results obtained can be explored in the form of 3D plots which explain the response in a visualized manner.

Fig.1: 3D response curve representing the surfactant concentration and stabilizer concentration effect on mean particle size

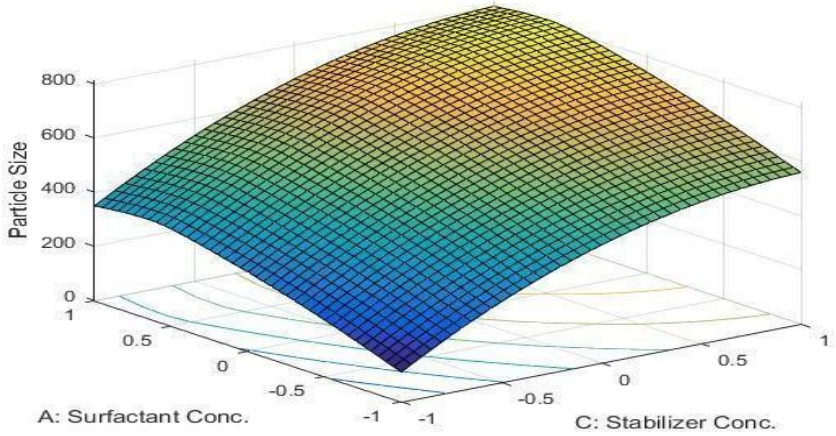


Fig.2: 3D response curve representing lipid ratio and stabilizer concentration effect on mean particle size

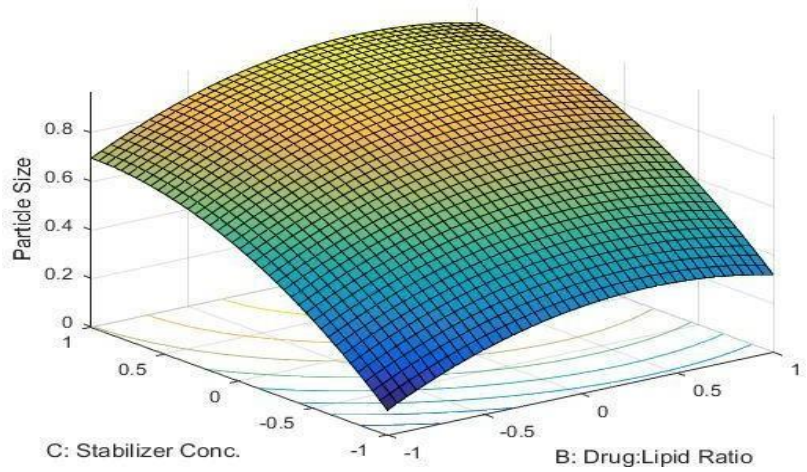


Fig.3: 3D response curve representing the surfactant concentration and lipid concentration effect on mean particle size

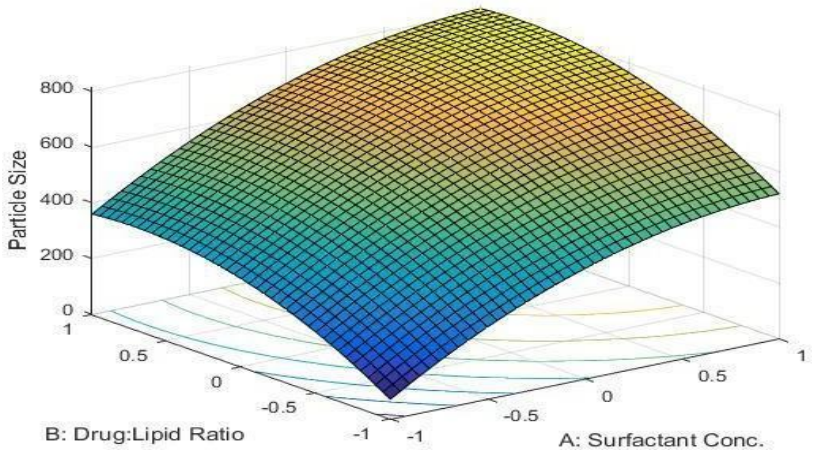


Fig.4: 3D response curve indicating the surfactant concentration and stabilizer concentration effect on PDI

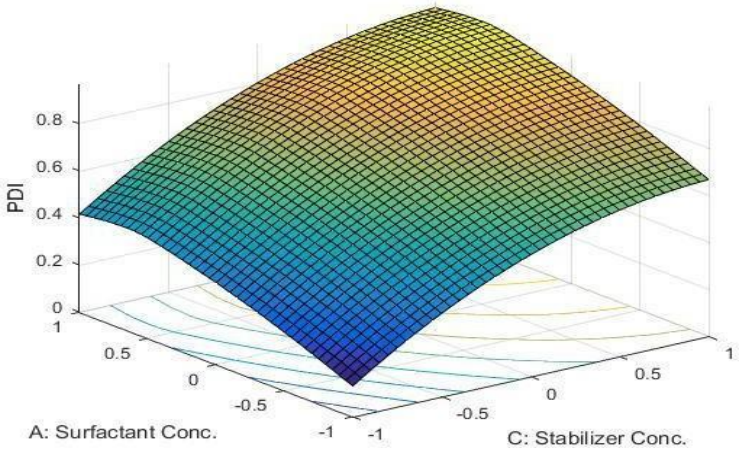


Fig.5: 3D response curve indicating the lipid concentration and stabilizer concentration effect on PDI

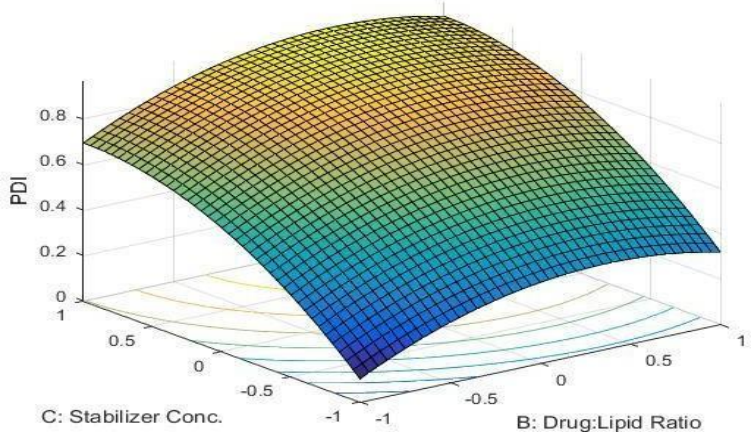
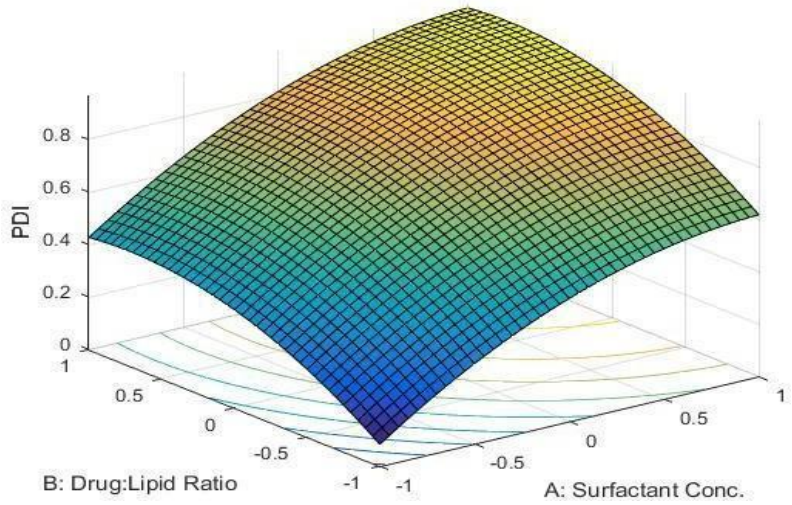


Fig. 6: 3D response curve indicating the surfactant concentration and lipid concentration effect on PDI



The derived results for optimum concentration of surfactant find 4% v/v of tween 80, the best suitable for emulsification purpose. The 1% w/v concentration of poly vinyl alcohol (PVA) is found adequate for stabilizer. The drug lipid ratio finds the value of 1:5 w/w with less particle size and higher entrapment efficiency. The particle size value predicted by the software was 150.4 nm and 0.189 for PDI. The experimental values were 151.1 and 0.214 respectively for particle size and PDI. These results support the optimization procedure of this study. The analysis of variance applied on the parameters represents the significant differences between the values for dependent variables. The regression analysis of data was performed using F test whose derived value of

3.35 in the test indicates the significance of model. R^2 value obtained 0.9172 indicates the significance of model. As per the statistical analysis, the batch GMS-9 is found the optimized batch for further studies among 27 possible combinations of drug- lipid, surfactant and stabilizers concentrations.

Fig.7: Effect of RPM on Particle size

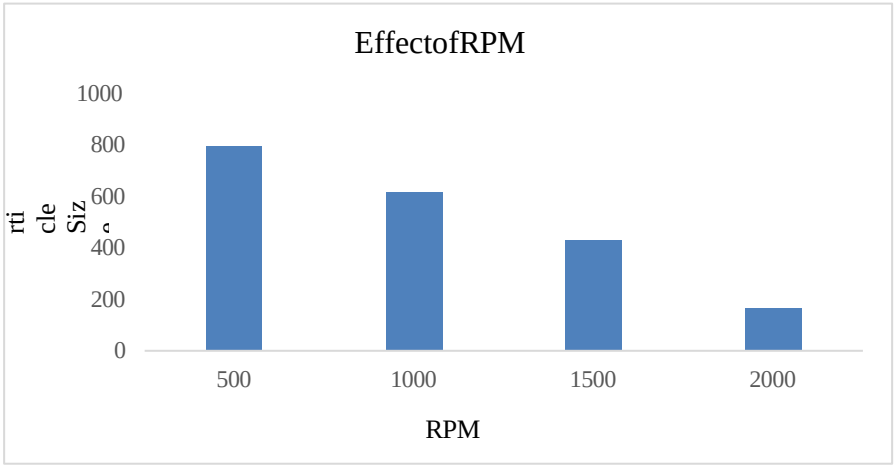


Fig.8: Effect of Time on Particle size

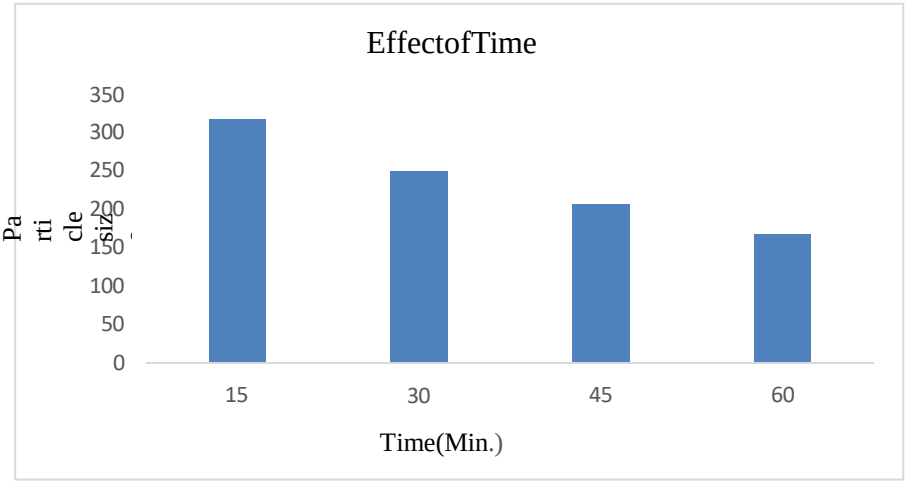
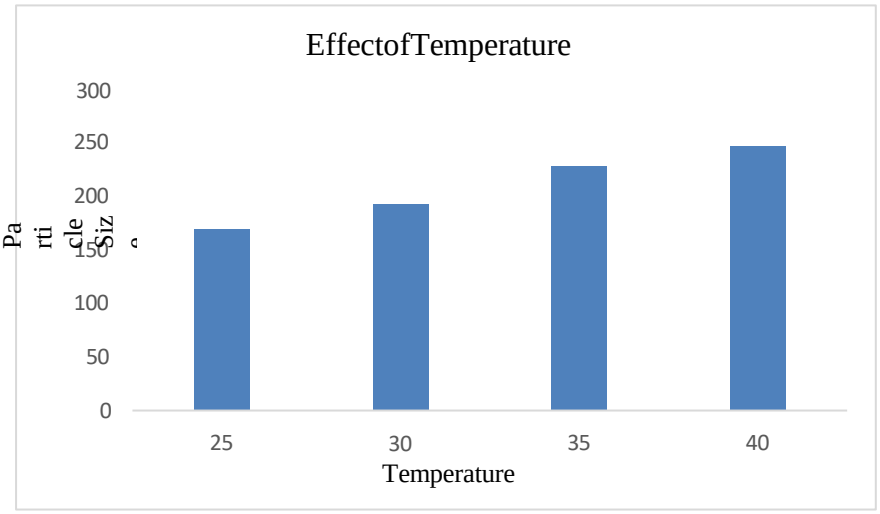


Fig.9: Effect of Temperature on Particle size



The process variables in the formulation development were determined by hit and trial method. The effect of revolution per time (rpm), time of stirring and temperature required to run the process were investigated. The effect of membrane filtration was also considered for process parameters. The effects of selected parameters were studied over particle size and size distribution of solid lipid nanoparticles.

Table 3: Selected parameters with their optimized values

Different variables in	**Parameters**	**Values after optimization**

	Concentration of emulsifier	4.0 % v/v
	Concentration of stabilizer	1%w/v
	Drug: lipid ratio	1:5 w/w

	Speed in rpm	2000 rpm
	Time (in min.)	60min
	Temperature (in °C)	Adequate temp. (25°C)
Process	Filtration	With membrane filtration

Table 4: % Entrapment efficiency, % drug loading and % yield for optimized Formulation (n=3)

Optimized formulation	**%Entrapment efficiency**	**Drug loading**	**% Yield**

GF-SLN- Itriconazole loaded solid lipid nanoparticles, GMS-Glyceryl monostearate

Table 5: Particle size, zeta potential and polydispersity index of optimized formulation (n=3)

Optimized batch formulation	**Mean size of Particles (nm)**	**Polydispersity Index (PDI)**	**Zetapotential (mv)**
GF-SLN(GMS-9)	151.1±10.7	0.172 ±0.07	-40.3±0.8

Fig.10: Scanning Electron Microscopy (SEM) image of Itriconazole loaded solid lipid nanoparticles

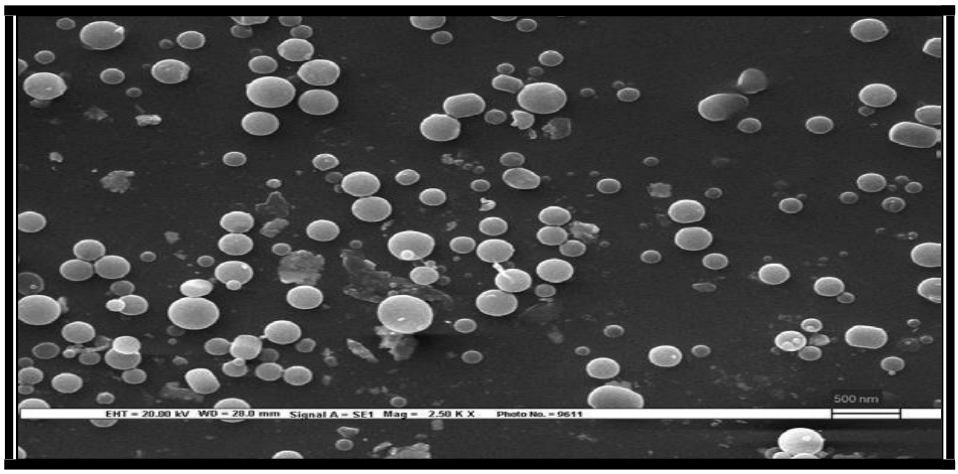
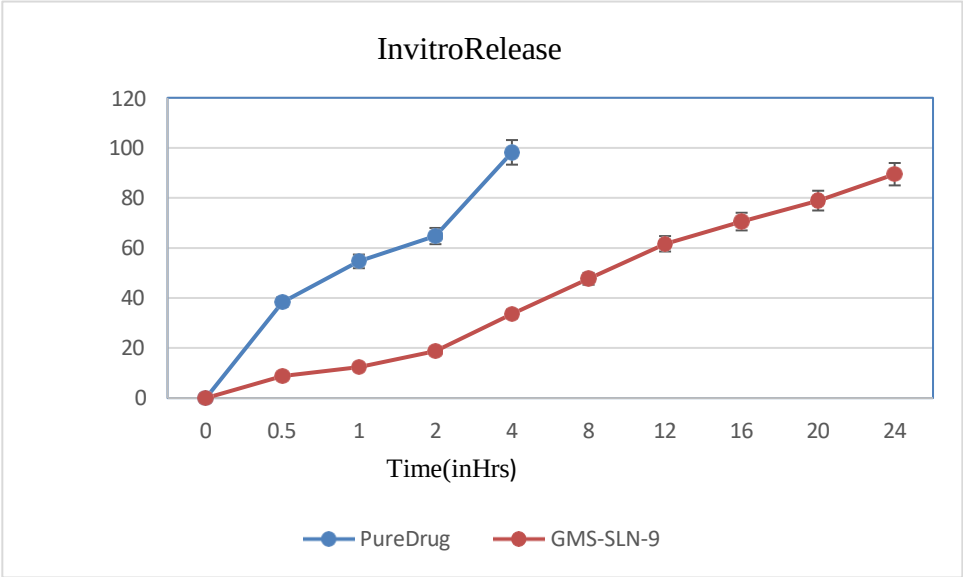


Fig.11: In-vitro release pattern showing release profile of pure drug suspension and Itriconazole loaded SLNs



The in-vitro studies concluded that the maximum release (98.23%) for pure drug suspension was obtained in 4 hrs. Solid lipid nanoparticles exhibited extended release of drug with release of 33.64 % drug in 4 hrs. The initial burst release from solid lipid nanoparticles was observed which may be due to deposition of certain amount of drug at the surface of developed SLNs. On subsequent studies, the drug containing solid lipid nanoparticles exhibited a sustained drug release. The cumulative percentage of drug released was 89.47% for a time period of 24 hrs.

Table 6: Storage conditions effect over particle size, % EE and zeta potential of Itriconazole loaded solid lipid nanoparticles

Cumulative Percentage Release

Temperature for storage (°C)	Duration of storage	Particle size (in nm)	Zeta potential (in mV)	% entrapment efficiency
5	0 day	155.41	-42.6	92.34
	2 months	156.32	-42.4	91.12
	4 months	159.23	-42.1	88.77
	6 months	164.83	-41.8	88.35
25	0 day	156.72	-41.23	91.27
	2 months	159.89	-40.56	89.56
	4 months	163.23	-38.23	88.28
	6 months	168.21	-38.56	88.21

CONCLUSION

The current research proved that the SLNs is a potential method for improving the biopharmaceutics properties of poorly water soluble drug i.e. Itriconazole (BCS class II) and open up new ideas for the formulation of drugs with low aqueous solubility and high permeability. The solid lipid nanoparticles containing Itriconazole was optimized based on lower particle size with less heterogeneity. Due to significant entrapment of lipophilic drug into SLNs, it exhibit controlled release of drug over a long period of time with improved bioavailability.

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