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OPTIMIZATION OF PROCESSING PARAMETERS FOR FORMULATION OF NANOSUSPENSION CONTAINING POORLY WATER SOLUBLE ANTIFUNGAL AGENT

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Abstract: A substantial portion (40%) of drugs fail full development, because of *Poor water solubility of drug molecules, insufficient bioavailability, fluctuating plasma levels and high food dependency. Itraconazole is one of such agent, a poorly water soluble drug indicates insufficient bioavailability following oral administration. The purpose of the present study was to formulate and optimize Itraconazole nanosuspension. In the present work Itraconazole nanosuspension was prepared by pearl milling technique using zirconium oxide beads as a milling media,* ,HPC LF as a polymeric stabilizer and sodium dodecyl sulphate as a sufactant. Initially various ratio of drug: polymer: surfactatnt were checked and optimized. Later on e*ffects of various process parameters like stirring time, amount of milling media and the ratio of the beads as milling media were studied on optimized ratio.* The optimized nanosuspension was lyophilized using mannitol (1:1 ratio) as a cryoprotectant.. *Nanosuspension was characterized by particle size and size distribution, drug content, scanning electron microscopy, differential scanning colorimetry and its dissolution profile was compared with marketed product. Optimized nanosuspension showed spherical shape with surface oriented surfactant molecules and a mean particle diameter of 305 nm. There was no significant change in crystalline nature after formulation and it was found to be chemically stable with high drug content. The in vitro dissolution profile of the optimized formulation compared to the pure drug and marketed formulation (Canditral Capsule) by using 0.1N Hydrochloric acid as release medium showed higher drug release.*

Keywords: Nanosuspension, Itraconazole, media milling technique, polymeric stabilizer

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INTRODUCTION

Plenty of new as well as existing drug substances are only very slightly soluble or even practically insoluble. A considerable portion (40%) of these drugs fail full development, because of Poor water solubility of drug molecules, insufficient bioavailability, fluctuating plasma levels and high food dependency[1-3].Major efforts have been made for the development of customized drug carriers to overcome the disappointing in vivo fates of the drug. Hence, there is a growing need for a unique strategy that can tackle the formulation related problems associated with the delivery of hydrophobic drugs in order to improve their clinical efficacy and optimize their therapy with respect to pharmacoeconomics.[4-7]

The dissolution rate of poorly water soluble-drugs often becomes a rate-limiting step in their absorption from GI tract. [8] Various solubilization methods have been used to increase the drug solubility and dissolution properties, including the use of surfactant, water-soluble carriers, polymeric conjugates and solid dispersions.[9-13] Recently nanonisation has become a popular approach since it is cost effective and technically simpler alternative, particularly for poorly soluble drugs, and yield a physically more stable product than liposome dispersions. With this technique, the drug, dispersed in water, is grounded by shear forces to particles with a mean diameter in the nanometer range (100-1000nm). The smaller particle size of the dispersed particles causes them to dissolve more quickly owing to their higher dissolution pressure and leads to an increased saturation solubility. This may enhance the bioavailability of drugs compared with other microparticular systems. If in vivo dissolution velocity of the drug particles is low enough, the drug nanosuspensions will have the passive targeting advantages of colloidal drug carriers. [14-17]

In present study Itraconazole was selected as it is practically insoluble in water, but possesses high permeability, which makes it an excellent candidate for nanogrinding Itraconazole is an orally active triazole antimycotic agent, which is active against a broad spectrum of fungal species including *Cryptococcus*, *Candida*, *Aspergillus*, *Blastomyces* and *Histoplasma capsulatum* var. *capsulatum* . It is a weak basic drug which is soluble in lipids (n- Octanol/Water partition, 5.66 at pH of 8.1) and a pKa of 3.7. Itraconazole is ionized only at a low pH, such as gastric juice and as a result on oral administration, the gastric acidity is required for adequate dissolution.[18-22]

The aim of this work was to formulate itraconazole nanosuspension by media milling technique for oral administration, enabling to enhance the saturation solubility, dissolution rate of itraconazole. Additionally influence of various processing parameters were also studied. The optimized nanosuspension formulation was evaluated for in vitro dissolution profile in comparison to the pure drug and marketed formulation. (Canditral Capsule).

Materials and methods:-

Materials :-

Itraconazole was a gift from Intas pharmaceutical limited (India). Zirconium oxide beads were gifted from Sun Pharmaceutical Industries Ltd. (India). Sodium dodecyl sulfate [SDS] (Crystal Chemicals Limited, Himmatnagar), Hydroxypropylcellulose [HPC-LF](Crystal Chemicals Limited, Himmatnagar. All chemicals and solvents used were of analytical grade.

Preparation of nanosuspension:-

For nanogrinding of itraconazole, solutions of surfactant and polymer stabilizers in purified water were first prepared. Itraconazole was then dispersed in the stabilizer solution. The ratio was selected based on previous experiment. (Drug:Polymer:Surfactant-20%w/v:5%w/v:0.3875%w/v).The resulting coarse pre-dispersion was comminuted using zirconium oxide beads (milling media) on a REMI Digital magnetic stirrer at higher speed. Zirconium oxide beads were used in the preparation of nanosuspension due to their low cost and easy availability for lab scale production of nanosuspension in comparison to silver beads.

Optimization of processing variables:

1) Influence of milling time and milling speed was determined on resultant optimized formulation using 3^2 factorial design.

2) Effect of ratio of beads as milling media was also checked for optimized formulation for specified milling time and speed.

The optimized processing parameters were further used to formulate nanosuspnesion for further characterization.

Characterization of nanosuspension :-

Particle size and Size distribution

The average particle diameter and size distribution of the prepared nanosuspension was determined by laser diffraction technique using Malvern particle size analyzer, SM 2000. Nanosuspension was added to the sample dispersion unit, and stirred at 2000 rpm with magnet in order to reduce the interparticulate aggregation and laser obscuration range was maintained between 10-20%. The mean particle size was measured by repeating the experiment in triplicates. The influence of stirring time and varying ratio of different size of beads on particle size were also determined.

Scanning Electron Microscopy (SEM)

The lyophilized powder for nanosuspension formulation was set aside in the sampling unit as a thin film and then photographs were taken at 100X and 200X magnification using Jeol Scanning Electron Microscope.

Differential Scanning Calorimetry (DSC)

The DSC thermograms of bulk Itraconazole powder and lyophilized nanosuspension formulation were taken on a DSC-60 differential scanning colorimeter between 30-300°C at a heating rate of 10°C/min with Nitrogen supply at 50.0 ml/min.

Drug Content

Assay was carried out by taking 10 mg of lyophilized powder (weigh equivalent to 1.25 mg of drug), dissolved in 0.4 ml of methanol in 50 ml dry volumetric flask and then volume was made up using 0.1 N HCl. Then 4 ml of the solution was taken to 10 ml dry volumetric flask, and volume adjusted with 0.1 N HCl. The absorbance at 254.0 nm wavelength was taken using SHIMADZU UV-VISIBLE spectrophotometer UV-1601 and the drug content was calculated accordingly. [19,21]

In vitro dissolution profile

In vitro dissolution study was carried out using USP dissolution test apparatus-I (basket assembly). The dissolution was performed using 900 ml of 0.1N HCl as dissolution mediums maintained at 37 ± 0.5°C and 75 rpm for pure itraconzole, lyophilized itraconazole nanosuspension formulation and marketed formulation (Canditral capsule). Samples (5ml) were withdrawn at regular intervals of 5 min for 90 min and replaced with fresh dissolution medium. Samples taken were filtered through 0.2µ filter paper and assayed spectrophotometrically on SHIMADZU UV-VISIBLE spectrophotometer UV-1601 at 254.0 nm wavelength. Dissolution for each formulation was performed in triplicates and mean of absorbance was employed to calculate cumulative percent of drug release (19,21).

Result and discussion:

Influence of processing parameters on optimized formulation :

1) **Effect of milling speed and milling time:** To determine effect of time and speed of milling, 3^2 factorial design was employed as described in table 1.

Table 1: coded value for determining effect of milling time and speed

Coded values	Actual values	
	X_1	X_2
-1	750	16
0	1000	20
+1	1250	24

Where, X_1 –Milling speed (rpm) X_2 - Milling time (hr)

Table 2: 3^2 factorial design layout for preparation of Itraconazole nanosuspension.

Batch No	X1	X2
IMF1	-1	-1
IMF2	-1	0
IMF3	-1	+1
IMF4	0	-1
IMF5	0	0
IMF6	0	+1
IMF7	+1	-1
IMF8	+1	0
IMF9	+1	+1

The results obtained are given in table 3.

Table 3: Optimization of milling parametes of itraconazole suspension

Batch No.	Milling Speed (rpm)	Milling time (hr)	Average Particle size	Polydispersity index
IMF1	-1	-1	0.545µm	0.527
IMF2	-1	0	0.516µm	0.502
IMF3	-1	+1	0.496µm	0.485
IMF4	0	-1	0.479µm	0.389
IMF5	0	0	0.375µm	0.325
IMF6	0	+1	0.325µm	0.410
IMF7	+1	-1	0.301µm	0.402
IMF8	+1	0	0.288µm	0.430
IMF9	+1	+1	0.242µm	0.494

The results of optimization are indicated below.

Table 4 : Fit Statistics for Y1

		Master Model	Predictive Model
Mean		0.396333	0.396333
R-square		96.17%	84.98%
Adj.	R-square	89.77%	82.83%
RMSE		0.036352	0.047102
CV		9.171996	11.88437

Predictive Model for Y1

Coded Levels(-1,1):

Y1 = 0.396333 - 0.121*X1

Uncoded Levels:

Y1 = 0.880333 - 0.000484*X1

Table 5: Fit Statistics for Y2

	Master Model	Predictive Model
Mean	0.440444	0.440444
R-square	94.62%	73.25%
Adj. R-square	85.65%	64.33%
RMSE	0.024918	0.039288
CV	5.657555	8.920106

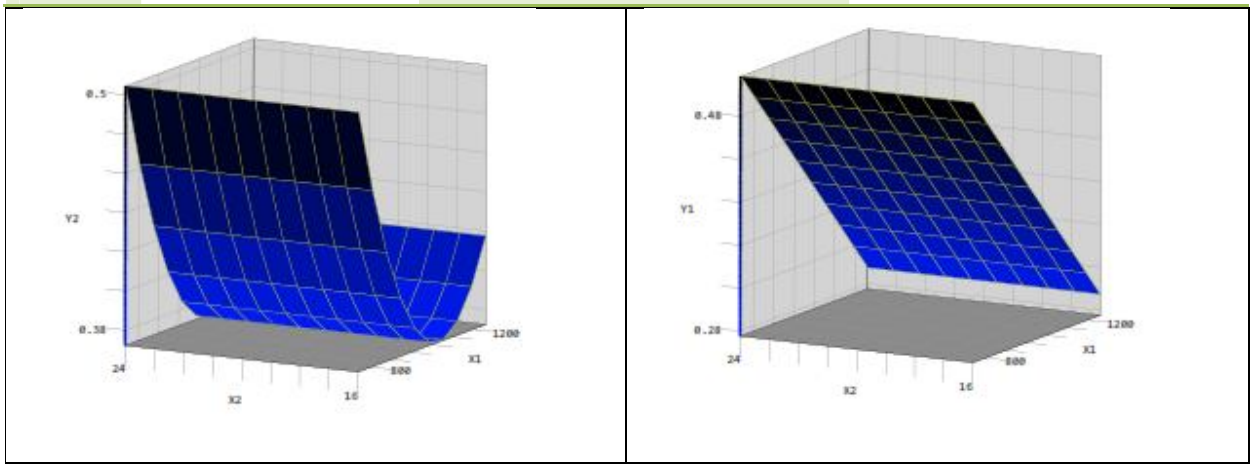

Figure 1 : Surface plots for Y1 & Y2:-

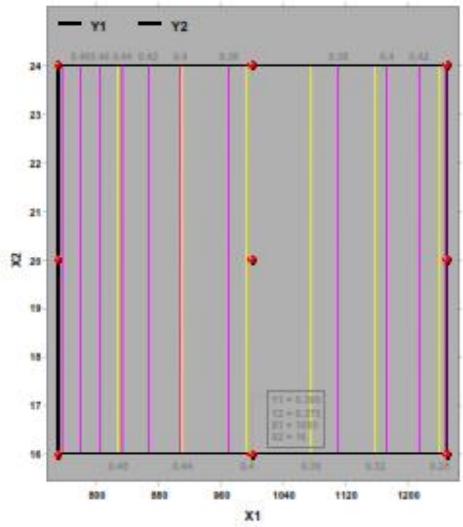

Figure 2: Contour plot for optimization

The results of optimization clearly indicate that the speed of milling is having more significant effect than the milling time. Additionally the check point batches had been taken to verify the results.

Table 6: Check point batch data: -

Coded		Uncoded		Predicted		Actual	
X1	X2	X1	X2	Y1	Y2	Y1	Y2
0.5	0.5	1125	22	0.336	0.384	0.326	0.379
-0.5	-0.5	875	18	0.457	0.415	0.448	0.421

The check point batches were again treated with chi^2 test to find out whether there is a significant difference or not for Y1 and Y2.

Table 7 : Resultant P value obtained from chi^2 test

Response	P Value obtained from chi^2 test
Y1	0.982614
Y2	0.990168

The data clearly stated no significant difference since both the values are far higher than 0.05.

Influence of ratio of various size of milling medium:-

Table 8: Effect of Ratio of beads for the preparation of Itraconazole nanosuspension

Batch. No.	Ratio of beads (Zirconium Oxide)		Mean particle size
	Small Size (0.4mm to 0.7mm)	Big Size (1.2mm to 1.7mm)	
ISB1	0	100	**1.102µm**
ISB2	25	75	**0.668µm**
ISB3	**50**	**50**	**0.303µm**
ISB4	75	25	**0.765µm**
ISB5	**100**	**0**	**1.215µm**

It is obvious from the data given in table 8 that equal ratio (50:50) of Small Size (0.4mm to 0.7mm) and Big Size (1.2mm to 1.7mm) beads had given least particle size. So the 50:50 ratio had been taken for formulation. The final formulation and processing parameters are given in table 9.

Table 9. Final formulation and parameters for characterization

Itraconazole	**20% w/v**
HPC LF	5 % w/v
SDS	0.3875 %w/v
Milling speed (rpm)	1000
Milling time (hr)	16
Ratio of beads (Smaller: larger)	50:50

Characterization of nanosuspensions

Particle size: The average particle size of optimized formulation was found to be 305 ± 5nm.

Scanning electron microscopy: The photomicrograph obtained are given in figure 3. it was clearly seen that the nanoparticles of the suspension had be spherical in shape compared to the bulk powder.

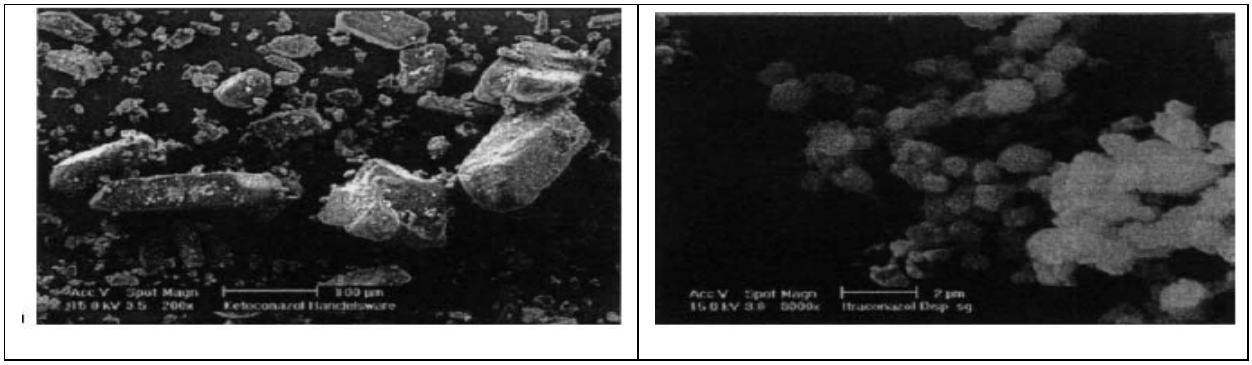

Figure 3.SEM photomicrograph of Itraconazole bulk and optimized nanosuspension.

DSC: The thermograms obtained clearly stated no interaction because there was no major changes in the temperature range thus no interaction occurred between drug and polymers.

pH: The pH of Optimized nanosuspension was pH 7.7±1.

Assay: Assay was found to be 99.25% in case of optimized formulation.

In vitro dissolution study: Drug release from pure drug, marketed formulation (Canditral capsule) and optimized nanosuspension formulation was determined by dissolution study.

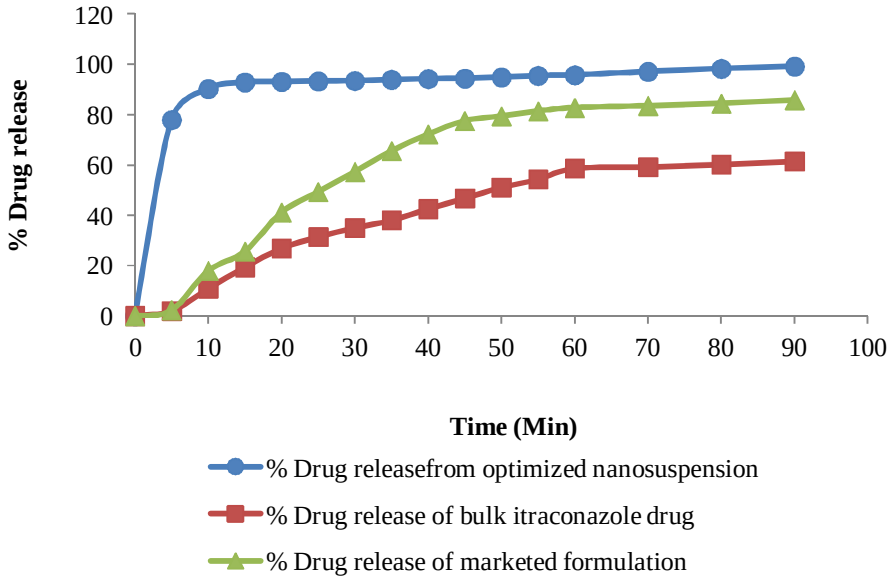

Figure 5: Dissolution profile for nanosuspension formulation (circle), pure drug (square), and marketed formulation (triangle) [mean ± SD (n=3)] in 0.1 N HCl.

The amount of drug released from the optimized nanosuspension formulation was 90% within 10 min in comparison to amount of 10% and 17% for pure drug and marketed formulation (Canditral capsule) respectively (Figure 5) in 0.1N HCl (pH 1.2). The increase in accessible surface area to the dissolution medium and hydrophilic polymer and surfactant coating on the particle surfaces may be considered as the core reason for approximately six fold increase in dissolution rate in optimized formulation.

CONCLUSION:

It may be concluded from the results of this study that nanocrystalline suspensions of poorly soluble drugs such as itraconazole can be easily prepared and lyophilized for extended period of time and represent a promising new drug formulation for oral drug delivery for treatment of fungal infection. Dissolution study in 0.1N HCl indicates that nanosuspension formulation gives higher drug release compared to the pure drug and marketed formulation. Moreover,

nanosuspensions represent a promising alternative to current delivery systems aspiring to improve the biopharmaceutic performance of drugs with low aqueous solubility.

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